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The Analysis of Human Urines for Steroid Substances[1]

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THE increasing attention given to the excretion of steroid substances in health and disease justifies the elaboration of analytical methods which will distinguish the various types of substances found in urine. Among the urinary steroids are biologically active estrogens, androgens and cortins. In addition there are physiologically inactive substances which in some cases are a more reliable index of hormone secretion than is the biologic activity found in urine. The present paper summarizes attempts to develop microchemical methods suitable for rapid, routine use, which permit segregation of the principal types of steroids and their quantitative determination.

In Table I are listed the identified steroid substances present in the urine of normal men and non-pregnant women (30). Dobriner *et al.* (7) have isolated small amounts of four crystalline ketonic steroids which they find regularly in normal human urines after acid hydrolysis. All except cortin are ordinarily obtained from acid-hydrolized urine. The quantitative variation of these substances forms the basis for numerous studies of endocrine function and hormone metabolism.

COLLECTION AND PRESERVATION OF URINE

It is important, especially for cortin extraction, to have fresh, well preserved urines. For this reason collections previous to extraction are made for no more than 48 hours and the urines are kept in twenty-four hour lots in a refrigerator at 5° C. Toluol (5 cc.) is added to each bottle before collection.

Since it has been demonstrated that there is a diurnal rhythm in the output of the 17-

[1] Investigations described in this paper have been aided by grants from the National Research Council Committee for Endocrinology, the Josiah Macy, Jr. Foundation and G. D. Searle and Company.

ketosteroids (23, 26), a number of our studies have been concerned with the nature of this phenomenon and its variations in health and disease. The routine procedure for urine collections for such studies is to obtain three specimens during the twenty-four hour span. The first covers the period of sleep (including night awakenings, which are noted on the specimen bottle). Initially urine is voided (but not collected) immediately before retiring, and the exact time noted on the label; all subsequent voidings to the time of waking are collected in this "sleep specimen."

The second specimen includes the period between waking and some three to four hours later, but is designated the "waking specimen." The third bottle contains all urine voided thereafter until time of retirement, and is designated the "day specimen." In normally active individuals, on an hourly output basis, the sleep specimen contains the least amount of 17-ketosteroid, the waking specimen, the most, and the day specimen, an intermediate amount. By making such a series of collections for three or four successive days the ordinary variations in output for any given individual can be observed.

TABLE I. STEROID SUBSTANCES REGULARLY FOUND IN HUMAN URINE

Name	Class of compound	Type of hormonal activity	Source of urine
Estradiol	Phenolic estrogen	Folliculoid	Pregnant female,[1] normal male,[2] non-pregnant female.[2]
Estrone	Phenolic estrogen	Folliculoid	Pregnant female,[1] normal male,[1] non-pregnant female[2]
Estriol	Phenolic estrogen	Folliculoid	Pregnant female,[1] normal male,[2] non-pregnant female[2]
Androsterone	Neutral 17-ketosteroid	Testoid	Normal male,[1] eunuch,[1] normal female,[1] ovariectomized female,[1] pregnant female.[1]
Aetioallocholanolone	Neutral 17-ketosteroid	Inactive	Normal male,[1] eunuch,[1] normal female,[1] ovariectomized female.[1]
Isoandrosterone	Neutral 17-ketosteroid	Testoid	Normal male,[1] normal female.[2]
Dehydroisoandrosterone	Neutral 17-ketosteroid	Testoid	Normal male,[1] eunuch,[1] normal female,[1] ovariectomized female.[1]
Androstenone-17	Neutral 17-ketosteroid	Testoid	Normal male,[1] normal female.[1]
Androstadienone-17	Neutral 17-ketosteroid	Inactive (?)	Normal male,[1] normal female.[1]
Pregnanediol	Neutral pregnane derivative	Inactive	Normal male,[1] normal female,[1] pregnant female,[1] ovariectomized female.[1]
Cortin	Neutral active fraction not yet chemically defined	Corticoid	Normal male,[2] normal female[2]
Cholesterol	Neutral, cholestane derivative	Inactive	Normal male,[1] normal female,[1] pregnant female.[1]

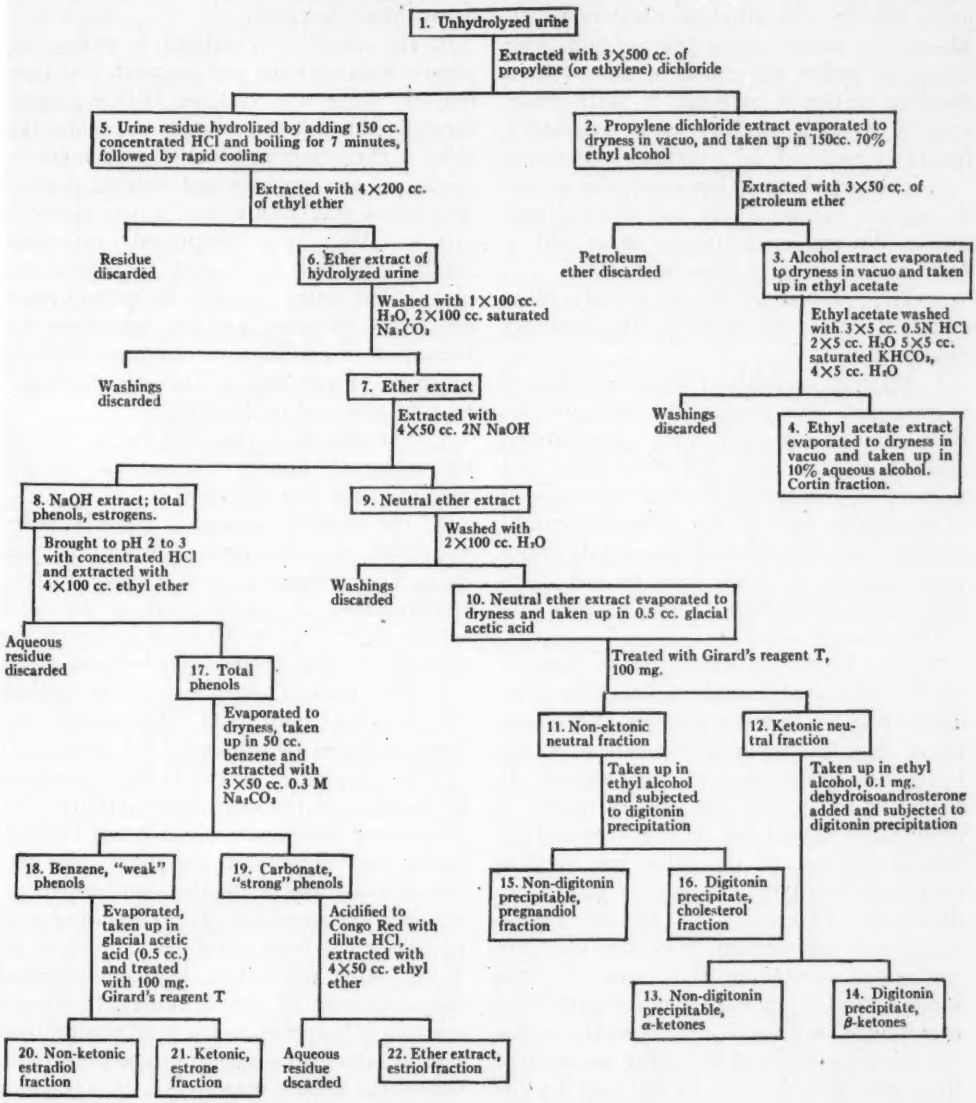
[1] Isolated.
[2] Indicated.

An aliquot is removed for creatinine determination from each urine specimen collected. Any gross errors in collection are revealed by departures in creatinine output from the individual's characteristic norm.

EXTRACTION OF URINE

In Table II a flow sheet is presented which outlines the method of fractionating the typical steroid substances in urine. Depending on the purposes of the study certain procedures may be omitted. We have numbered each principal step, and present below

TABLE II. FRACTIONATION OF URINARY STEROIDS[1]


1. Unhydrolyzed urine
Extracted with 3×500 cc. of propylene (or ethylene) dichloride
5. Urine residue hydrolized by adding 150 cc. concentrated HCl and boiling for 7 minutes, followed by rapid cooling
2. Propylene dichloride extract evaporated to dryness in vacuo, and taken up in 150cc. 70% ethyl alcohol
Extracted with 4×200 cc. of ethyl ether
Extracted with 3×50 cc. of petroleum ether
Residue discarded
6. Ether extract of hydrolyzed urine
Petroleum ether discarded
3. Alcohol extract evaporated to dryness in vacuo and taken up in ethyl acetate
Washed with 1×100 cc. H2O, 2×100 cc. saturated Na2CO3
Ethyl acetate washed with 3×5 cc. 0.5N HCl 2×5 cc. H2O 5×5 cc. saturated KHCO3, 4×5 cc. H2O
Washings discarded
7. Ether extract
Washings discarded
4. Ethyl acetate extract evaporated to dryness in vacuo and taken up in 10% aqueous alcohol. Cortin fraction.
Extracted with 4×50 cc. 2N NaOH
8. NaOH extract; total phenols, estrogens.
9. Neutral ether extract
Washed with 2×100 cc. H2O
Brought to pH 2 to 3 with concentrated HCl and extracted with 4×100 cc. ethyl ether
Washings discarded
10. Neutral ether extract evaporated to dryness and taken up in 0.5 cc. glacial acetic acid
Aqueous residue discarded
17. Total phenols
Treated with Girard's reagent T, 100 mg.
Evaporated to dryness, taken up in 50 cc. benzene and extracted with 3×50 cc. 0.3 M Na2CO3
11. Non-ektonic neutral fraction
12. Ketonic neutral fraction
Taken up in ethyl alcohol and subjected to digitonin precipitation
Taken up in ethyl alcohol, 0.1 mg. dehydroisoandrosterone added and subjected to digitonin precipitation
18. Benzene, "weak" phenols
19. Carbonate, "strong" phenols
15. Non-digitonin precipitable, pregnandiol fraction
16. Digitonin precipitate, cholesterol fraction
Evaporated, taken up in glacial acetic acid (0.5 cc.) and treated with 100 mg. Girard's reagent T
Acidified to Congo Red with dilute HCl, extracted with 4×50 cc. ethyl ether
13. Non-digitonin precipitable, α-ketones
14. Digitonin precipitate, β-ketones
20. Non-ketonic estradiol fraction
21. Ketonic, estrone fraction
Aqueous residue dscarded
22. Ether extract, estriol fraction

[1] The amounts of reagents indicated are for one liter volumes of urine.

requisite details. It should be stressed that the reagents used throughout are c.p. (except as otherwise indicated). All organic solvents are redistilled. Ethyl ether is rendered peroxide-free by shaking with ferrous sulfate before redistilling. Distilled water is used for all water washes.

1. The removal of "free" cortin by organic solvents from human urines has been accomplished ordinarily by an ethylene dichloride extraction from slightly acidified urine (8, 43). The tendency to form emulsions exhibited frequently by ethylene dichloride is avoided by the use of propylene dichloride.

Corticoid substances, however, partition more readily into ethylene dichloride (20, 32) and the higher boiling point of propylene dichloride makes the choice of solvent here chiefly a matter of convenience. With either solvent vigorous shaking in a separatory funnel is required for maximal extraction.

2. The petroleum ether extraction serves to remove various lipids and urinary pigments. We employ petroleum ether with a boiling-point range of from 30° to 60° C.

3. The removal of various urinary bases and acids is accomplished by the washings described here.

4. Material insoluble in 10 per cent ethanol is discarded. This extract is satisfactory for cortin assay, especially when administered orally to test animals. The extractions used to this point are chiefly to effect the removal of substances toxic to the adrenalectomized animals. It has been found that certain urines yield extracts which are toxic on test injection, while others do not. Such toxicity prevents accurate assay.

5. The hydrolysis employed is that of Smith (36) except that the boiling time is reduced from ten to seven minutes. It has been found that this reduces to a minimum the hydrolytic destruction of free steroid. It should be noted that this urinary residue is presumably devoid not only of free corticosteroid but also of any other free steroids readily removed by ethylene or propylene-dichloride. Practically all of the steroid substances represented here are therefore conjugated substances. If removal of cortin is not desired or if a more complete extraction of estrogens, androgens, etc., is wanted, steps 1 to 4 are omitted and the entire specimen of fresh urine is hydrolized. In this case if a distinction between "free" and "bound" material is desired, extraction of the unhydrolized urine (brought to pH 4 to 5) with ethyl ether is carried out. Ethyl ether is the solvent of choice for 17-ketosteroids (5, 24). In fact Eggleston *et al.* (9) have successfully employed this solvent for cortin extraction with the aid of a continuous extraction apparatus. If continuous ether extraction is employed special precautions against the explosion hazard must be taken.

6. The ethyl ether extract is washed to remove organic acids and pigment. The preliminary wash with distilled H_2O is poured carefully through the solution and along the sides of the separatory funnel, with a gentle mixing of the aqueous and organic phases. Emulsions may result from a too vigorous mixing. We have employed saturated $NaHCO_3$ as well as Na_2CO_3 to achieve removal. The latter "cleans" the extract more effectively, however, and also minimizes the formation of emulsions in the next step. As much as 10 per cent of the estriol present may be removed by Na_2CO_3.

7. The phenols in the ether extract are removed by 2N NaOH. The neutral steroids remain in the ether solution.

8. The phenolic estrogenic material is reextracted into an organic solvent, at this stage. The estrogens are now ready for further fractionation. If measurement of the total estrogenic activity of the urine is desired the assay of the total phenols may be carried out.

9. The neutral ether extract is washed free of alkali with H_2O. This extract has been employed by others for the determination by colorimetry of the 17-ketosteroids or by bioassay of the androgenic activity. The presence of chromogen which is not 17-ketosteroid has necessitated the use of color correction equations when the Zimmerman reaction is employed (12, 41). The use of such equations has been questioned, however (4, 5, 24). Salter and Cahen (34) have indicated the usefulness of the antimony trichloride reaction (22) applied to the total neutral fraction. A safer course appears to be the separation of the ketonic material (4, 28, 42) which is described in subsequent steps.

10. This is the micro-Girard reaction essentially as described previously (28). The reaction involves heating at 100° C. for 20 minutes. The reaction mixture is cooled, 15 cc. of ice-cold distilled water is added in 5 cc. portions and the solution placed in a small separatory funnel that has been previously kept cold in a refrigerator or on a cracked-ice

bed. The cold solution is rapidly extracted with 3×20 cc. of cold ethyl ether. The ether removes non-ketonic material. The non-ketonic fraction is washed once with cold H_2O which is added to the bound ketonic residue. The latter is hydrolized by adding 3 cc. of concentrated HCl and allowing it to stand for at least two hours at room temperature. The ketones are then removed by 3×20 cc. of ethyl ether which is washed once with 5 cc. 5 per cent K_2CO_3 and twice with 10 cc. portions of H_2O. The non-ketonic extract is similarly washed. The ether in each fraction is evaporated.

11. Digitonin precipitation is accomplished by methods previously described (14, 19). The principal non-ketonic β-steroid thus removed is cholesterol. In the α-fraction the principal non-ketonic steroids hitherto described are pregnanediol and its α-isomers. This fraction from normal urines has not been completely explored (30); its androgenic activity is unknown. It contains, in addition to steroid substances, varying amounts of hydrocarbon. The hydrocarbon may be segregated by esterifying and removing the hydroxylated compounds. The reagents commonly used for such separation are phthalic anhydride and succinic anhydride. An appropriate micro-method has been described (28).

12. The ketonic neutral fraction may be used for the determination of total 17-ketosteroid by the application of the m-dinitrobenzene or antimony trichloride color reactions. If a comparison of the α-ketosteroids and β-ketosteroids is desired, then digitonin precipitation of the β-ketosteroids is carried out. Where small amounts of β-ketosteroid are suspected optimal precipitation is assured by adding 0.1 mg. dehydroisoandrosterone to the ketonic fraction. The present data indicate that regardless of the β-ketosteroid content, digitonin precipitation results in the loss of approximately 0.02 mg. of β-ketone to the α-ketone-fraction. Digitonin precipitation and separation of the two fractions is accomplished as follows:

To the aliquot taken up in 95 per cent ethanol in a 50 cc. centrifuge tube is added 0.10 mg. dehydroisoandrosterone (in 1 cc. absolute ethanol) followed by the addition of 1 cc. of a 1% digitonin in 90 per cent ethanol. The combined solution is evaporated in a water bath at 85° C. and brought to dryness in a vacuum desiccator over H_2SO_4 (for 5 min.). One cc. of 80 per cent ethanol is added, brought to a boil and stirred well with a thin rod in order to dissolve as much of the precipitate as possible. Usually the precipitate is completely dissolved. Ten cc. of anhydrous, peroxide-free ethyl ether is added in small portions, permitting the insoluble digitonides to flocculate before adding the final 6 to 8 cc. After centrifuging, the clear supernatant fluid is collected in a test tube and to it is added two, 7 cc. ether washes of the insoluble digitonides. The supernatants contain the α-fraction. Cloudiness in the supernatant fluid may be avoided by a second short, high-speed centrifugation (about 5 minutes); stirring of the precipitate during the ether washings should be minimized.

To the β-fraction (insoluble digitonides) 0.5 cc. of pyridine (dried over BaO) is added. The solution is warmed to 60°–70° C. and stirred until the precipitate is dissolved. Five cc. of ethyl ether is added sufficiently slowly to permit the digitonin to form a flocculant precipitate. After 10 to 15 minutes of centrifugation the clear supernatant fluid is decanted. The dissolving of the digitonin in pyridine, precipitation with ether, and collection of the supernatant fluid are repeated. The collected ether-pyridine solutions are evaporated to dryness in a water bath and treated in a vacuum desiccator over H_2SO_4 for about 30 minutes. This treatment should remove the last traces of pyridine. Where dehydroisoandrosterone has been added it is necessary to subtract 0.08 mg. from the assay value obtained.

13. This fraction contains the α-ketones. Among the 17-ketosteroids this includes, in normal urines, androsterone and aetiocholanolone as the principle constituents. Androstenone-17 is another constituent, with Δ3,5 androstadienone-17 as a minor com-

ponent. The latter two substances along with other non-alcoholic ketones may be separated from the hydroxylated ketones by succinic anhydride treatment (28). The application of the m-dinitrobenzene or the antimony trichloride color reaction gives a measure of the 17-ketosteroid content. In pregnancy urines the presence of 20-ketosteroids complicates the use of the Zimmerman reaction.

14. This fraction contains the β-ketones. Among the 17-ketosteroids this includes in normal urines isoandrosterone or dehydroisoandrosterone or both (6, 19). The application of the m-dinitrobenzene reaction is preferable here for measurement since the antimony trichloride reaction fails to react with dehydroisoandrosterone. The β-ketosteroids ordinarily form a minor fraction of the total ketosteroids (3). In pregnancy urines 20-ketosteroids are present in detectable amounts (21).

17. The total phenols may be separated into estriol (steps 19 and 21), estradiol (steps 18 and 20) and estrone fractions (steps 18 and 21). These separations have been previously described (29, 21). The wash with Na_2CO_3 solution will effectively remove as little as 10 micrograms of estriol from benzene. Similarly 10 micrograms of estrone may be separated by means of the micro-Girard reaction which may be applied here exactly as in step 10.

By the application of the foregoing procedures one emerges with eight fractions each segregating various types of urinary steroids. Since most of them are present in normal urines in microgram quantities it is necessary to have adequate collections for assembling a sufficient amount for a complete quantitative determination. Only the 17-ketosteroids may be determined accurately in as little as an hour's collection of urine. The quantitative limitations for the other steroids are indicated by the assay methods described below.

Before proceeding to a description of assay methods certain precautions usually taken with extracts should be noted. All extracts are stored in the dry state in well stoppered vessels. Certain fractions may be preserved in the cold in ethanol (95 per cent or absolute) for a week or more (*e.g.*, fractions 16, 20, 21, 22) or for a few days (*e.g.*, fractions 4, 13, 14, 15) but the safest procedure is to keep them dry until used for assay.

METHODS OF ASSAY

Estrogen

The estrogens (fractions 20, 21, 22) are assayed by injection into spayed rats by a modified Allen-Doisy method (27). With our colony of Sherman-strain rats a rat unit of estradiol is 0.125 microgram, and of estrone and estriol, 1.0 micrograms. The urinary estrogens of several men and women have been determined using urines collected during a period of several days. The data are presented in Table III. Cortin removal (steps 1 to 4) was not practised on these urines.

TABLE III. ESTROGEN-ASSAY VALUES FOR SEVERAL HUMAN URINES (IN RAT UNITS PER 24 HOURS)

Subject	Sex	Estradiol	Estrone	Estriol
1	Male	4.5	4.6	3.3
2	Male	3.2	3.0	1.4
3	Male	6.0	5.8	5.0
4	Female	50.0	58.5	46.7
5	Female	6.4	4.6	2.4

The shape of our dosage-response curves is such that moderately accurate determinations of estradiol can be made at the 0.10 microgram level, or estrone at the 0.40 microgram level, and of estriol at the 0.30 microgram level. Routinely we require a minimum of 16 rats for each assay which at the unit level (50 per cent positive) gives a standard error ($\pm\sqrt{pxq/n}$) of ± 12.5 per cent in per cent-positive determination. This error can be reduced, of course, by increasing the number of the assay animals, but a corresponding increase of extracted estrogen is requisite. Table III demonstrates that with 16 assay rats the ordinary amount of urine required represents about three days' collection for men and two days' for women. Of course, where the output of one or more fractions is rather low (*e.g. subject no. 2*, estriol) a longer collection is necessary, and we routinely require a 96 hour period of collection to have an adequate margin of safety. Normal women show variations in their excretion of these fractions at various stages of

the menstrual cycle (37, 38), (also compare *subjects 4* and *5*, Table III) and a notable reduction in output after the menopause (27, 29).

Pregnanediol

The color reaction with H_2SO_4 (40) is available for the estimation of pregnanediol in fraction no. 15, provided that more than 0.5 mg. is present, and precipitation to remove interfering material is practiced. If 10 mg. or more of pregnanediol is present it is possible to crystallize this substance out of fraction 15 and identify it by the melting point, but accurate quantitative determination is not possible.

Cholesterol

We have applied the Lieberman-Burchard color reaction (16) to determine the cholesterol content of fraction 16. Since certain individuals will excrete 0.01 mg. or less per 24 hours, (39) colorimetric estimation is likely to be only approximate, and cannot be applied to short-time collections.

The 17-Ketosteroids

Colorimetric estimation using the Zimmerman reaction may be employed either with the ketonic neutral fraction (no. 12) or with the α- and β-fractions (no. 13 and no. 14). We have employed the aqueous alcohol method (18), although we now incubate the reaction mixture at 28° C. for 90 minutes to allow full color development. Nathanson and Wilson recommend 105 minutes at 25° C. (17). The longer incubation time brings into closer agreement the somewhat varying color intensities exhibited following short incubation times by the various 17-ketosteroids in urine extracts (5, 17). Black (4) has observed that even with ketonic fractions of urine the typical 17-ketosteroid color is not always obtained in the Zimmerman reaction, and we have already noted that the 20-ketosteroids of pregnancy urine will cause an overestimation of 17-ketosteroid content (5, 28). (In the total neutral fraction there are in addition non-ketonic chromogens.) For this reason antimony trichloride (22) is applied to obtain more specific values.

Since dehydroisoandrosterone is only feebly chromogenic with antimony trichloride, its titer is minimized by this determination (22, 34). If present, it will be in the β-ketone fraction (no. 14) and can be estimated (along with other β-ketones present) by applying the Zimmerman reaction to the β-ketone fraction. Antimony trichloride applied to the α-ketone fraction will approximate rather accurately the 17-ketosteroid content since androsterone, aetiocholanolone, and androstenone all react typically.

Androgen Assay

We have conducted determinations of the androgen content of the ketonic neutral fraction (no. 12) of a number of urines. Topical application of the extracts to the chick's comb is employed.

White Leghorn cockerels, 48–60 hours old at the start of the experiment, are used. They are separated in groups of 10, every group in the same brooder being colored differently with appropriate dyes. The animals have continuous access to food until 12 hours before the results are observed and to water for the duration of the experiment. The temperature of the brooder is kept at 35° ± 2° C.

In our experience the androgenic activity in the ketonic neutral fraction is always less than the Zimmerman 17-ketosteroid titer (in androsterone equivalents) would imply. Accordingly we prepare a convenient stock solution containing 0.25 mg. of 17-ketosteroid per cc. in 95 per cent ethanol and remove test-samples as needed. The latter are dried and two samples (one containing twice the amount of the 17-ketosteroids in the other) are prepared for assay in 10 chicks each. The dried sample is taken up in ethyl ether containing 1 per cent mineral oil. The ether solutions are kept in small graduated tubes, stoppered with corks and kept in an ice-salt mixture for the duration of the experiment. Daily readings of volume are taken and any changes due to evaporation are corrected by adding the requisite amount of 1 per cent mineral oil in ether solution. The ether solution is applied topically to the comb; the dosage is 0.02 cc. per day for 7 days. A 0.2 cc.

pipette graduated in 0.01 cc. is used; the point is drawn to a capillary, and the application is made by touching the point to the comb and drawing it along the comb length.

Standard androsterone assays are conducted with each set of extracts, using usually three concentrations of androsterone (with our chicks 0.5, 1.0 and 2.0 micrograms per chick have proven satisfactory). The chicks are sacrificed on the eighth day, having been deprived of food after the seventh day. The combs are removed and weighed, the results being expressed in terms of the weight of the comb (in mg.) per gram of body weight. Extract values are determined in terms of androsterone (in micrograms) by comparison with the data on the androsterone standard.

Cortin

Various methods of bioassay have been proposed (34–36). We have attempted to employ the cold test (35, 45) but have found it too variable to detect accurately the small amounts of activity usually present in normal urines. The proposed liver-glycogen method of Venning *et al.* (44) and Olson *et al.* (18) appear to be promising for the assay of the corticoids that affect sugar metabolism. A bio-assay method which offers a basis for good quantitative determination is that recently described by Elmadjian (10). It is a stress test which involves a measure not only of cortins related to sugar metabolism, but also of desoxycorticosterone.

DISCUSSION

The method of analysis presented here affords a means of segregating and measuring four major classes of steroid substances: (a) the estrogens, (b) the neutral ketosteroids, (c) the neutral non-ketonic steroids, and (d) the urinary cortins.

The feasibility of fractionating the estrogens is indicated. The usefulness of such fractionation for research purposes has been demonstrated in numerous instances, (2, 27, 38). Even employed as a routine, this may yield valuable information. Several cautions against too strict an interpretation are necessary. Into the three major fractions estradiol, estrone, and estriol are segregated almost quantitatively; there is, however, a fractionation error of approximately 5 per cent for each step, and thereby as much as 5 per cent of the "weak" phenols (fraction 18) may be extracted with the "strong" phenol fraction (no. 19). Thus if α-estradiol and estrone are present in large quantities and estriol in a minor quantity, a notable activity of the estriol fraction may be due to estradiol. Again, the micro-Girard separation is of such a nature that a small percentage of estradiol may be included in the estrone fraction. A second caution is that the activity observed may not necessarily give a quantitative indication of the estrogens present. In certain urine extracts substances which inhibit or potentiate estrogenic activity in test-animals have been reported (11, 27). Colorimetric determination of the estrogenic concentration in late pregnancy urines has been accomplished with some success (1, 31), but in non-pregnancy urines (and male urines) the extracts contain large amounts of non-estrogenic chromogen, and even the ketonic which is usually the "cleanest" fraction gives overestimates of actual content by the colorimetric reactions usually employed (33).

The determination of the neutral 17-ketosteroids may be accomplished rather accurately, if the Girard separation is practiced. Fractionation of the neutral ketones may lead to more detailed information. It should be recalled, however, that the presence of substances other than 17-ketosteroids may lead to 17-ketosteroid overestimates (5, 13), if the Zimmerman reaction is employed. The use of antimony trichloride should reduce this error.

The usefulness of determining neutral non-ketonic steroids, pregnanediol and cholesterol, will depend on the particular problem involved. This urinary fraction probably also contains other steroid substances not hitherto described or identified. By employing a succinic anhydride treatment the hydrocarbons of urine may be isolated into the non-hydroxy fraction, and in the remaining alco-

holic fraction any investigation of typical steroids may be made. We have applied the $SbCl_3$ reaction (22) to this fraction, and found in it small amounts of an interesting chromogen which increased in amount after the administration of certain steroid hormones (25), but clinical correlations remain to be established.

The usefulness of cortin-excretion studies in clinical medicine is not established but interesting possibilities have been indicated (8, 43). The methods of extraction can probably be improved since a certain amount of "bound" cortin is probably present. The full development of accurate assay methods remain to be published, but an opportunity for some differentiation of corticoid type is afforded by the various methods being developed. Attempts to determine urinary cortin by spectrophotometric methods have been made in these laboratories, but thus far no usable technique has been evolved.

The efficiency of the separations employed has largely been dealt with elsewhere. Thus the partitioning of the estrogens between benzene and Na_2CO_3 has been adequately described by Mather (15), Bachman and Pettit (2) and Pincus and Pearlman (29). Similarly, we have indicated the efficiency of separation of neutral 17-ketosteroids by the micro-Girard reaction (28). Until the nature of the urinary cortin is better known, model recovery experiments are hazardous, but we have observed nearly quantitative recovery of adrenal extract added to urine and subjected to the procedures of Table II (20). As indicated in the course of this presentation most of the methods described have been thoroughly tested in one connection or another. In this paper we present a means for the concordant application of various adequate micromethods.

SUMMARY

Methods are described for the extraction and separation of urinary steroids into fractions allowing the estimation of substances usually present. The technique of quantitative assay of the various sustances by microcolorimetric or biological methods is indicated.

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The Estrogen Treatment of Stricture of the Rectum Due to Lymphogranuloma Venereum[1]

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CLINICAL observations noted accidentally may lead to new and important therapeutic conceptions. The treatment of stricture of the rectum due to lymphogranuloma venereum has, until the present time, been highly unsuccessful. The observation that a stricture of this type improves tremendously during pregnancy and renders the patient free from symptom after delivery stimulated the search for the explanation of this phenomenon. This report of four cases will serve to indicate the beneficial and almost specific effect of estrogens in alleviating rectal strictures of this type.

Lymphogranuloma inguinale or lymphopathia venereum (9) is a wide spread communicable disease usually acquired venereally. It affects all races but is especially common in the colored race. It occurs in both males and females and gains entrance through the skin and mucous membranes. The disease is believed to be due to a filterable agent (2) and is diagnosed by a positive Frei test (1) and a positive complement fixation test (8). The earliest accurate report of the disease was made by Wallace in 1833, although John Hunter first described the buboes in 1786. In the United States, Klotz (6) was the first to report 120 cases seen by him in New York between 1879 and 1889. In 1925, Frei (1) introduced the specific Frei test and in 1930 Hellerstrom (5) transmitted the disease to animals. Later Levaditi (7) used the mouse to demonstrate the antibodies to the virus.

In 1942 H. Goldman (3) was the first to suggest the use of hormones to simulate the pregnant state in the treatment of rectal stricture complicating lymphogranuloma venereum. Goldman's patient was a colored female, 25 years old, who was admitted to the Hospital for Joint Diseases in September, 1935, with rectal bleeding, anorectal discomfort and rectal stricture of 5 years' duration. She was advised to have a colostomy. The patient had a positive Frei test and a reversed albumin-globulin ratio. There was no improvement with Frei antigen treatment or other known treatment. She returned again in May 1940 with the rectal stricture as before and received large doses of the sulfanamide drugs and Frei antigen injections, without improvement. In December, 1940, she was four months pregnant, and it was noted that the stricture was becoming rapidly absorbed. In 1941 she was delivered by Caesarean section because of cephalo-pelvic disproportion. In January, 1942, she had no rectal complaints, her bowel movements were normal and no stricture was visualized. Palmer, Kirsner, and Radaniche (10) in 1942 observed a similar case, and the patient experienced relief during pregnancy, but they credited the disappearance of the stricture to

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the use of sulfonamides which were administered during the pregnancy.

Clinically, in the female, rectal involvement takes place by direct extension by way of the lymphatic vessels from the vagina to the rectum through the glands of Gerota. The stricture of the rectum is usually accompanied by a secondary proctitis. In one series this occurred in 92.5 per cent of the cases (4). The disease usually begins in the rectum as an ulcerative proctitis and then gradually develops into a stricture. The stricture causes restriction of the passage of feces, and is accompanied by abdominal cramps, distension, tenesmus and, in the past, this nearly always necessitated the performance of either a colostomy or an ileostomy for relief.

The diagnostic features of the disease are: (1) increased globulin in the blood, reversed albumin-globulin ratio; (2) positive Frei test; (3) leucocytosis and increased sedimentation rate; (4) occasionally, false positive Wassermann; (5) positive specific complement fixation test (which is 98% accurate).

The treatment in the past consisted of injections of Frei antigen and antimony compounds, the application of carbon dioxide snow, repeated dilatations, and colostomy. The use of the sulfonamides in the disease was introduced in 1938 and, combined with Frei antigen injections, had given the best results in the early stages. This treatment has proved of value in clearing up the ulcerative proctitis, but is of no value in the late rectal stricture stage. We decided to try to simulate the pregnant state in the treatment of stricture. First, one must consider the hormones involved. It is well known that the secretion and blood content of estrogens, progesterone and anterior pituitary gonadotropins are increased in pregnancy. Rather than try all three together it seemed advisable to utilize one at a time. Since progesterone is expensive and since there is no cheap and potent anterior pituitary extract, it was decided to try estrogens by mouth first. Using three to five times the therapeutic dose of estrogens ordinarily employed for other conditions very satisfactory results were obtained: the strictures cleared up in from three to five weeks. The estrogens used were parahydroxyphenyl ethyl hexane, hydroxyphenyl hexane, ethinyl estradiol, estrone sulphate and diethylhydroxymethoxystilbene or combinations thereof.

CASE REPORTS

Case 1. S. H., 56 years of age, appeared in the Gynecological Endocrine Clinic January 11, 1944, complaining of intermittent vaginal bleeding of 8 months' duration, and inability to move the bowels normally for many years. Physical examination revealed a suspicious lesion of the cervix which, on biopsy, proved to be benign. There was also a circular stricture of the rectum about 5 cm. above the anal orifice. This stricture was so narrow that it did not admit a probe. The patient was admitted to the hospital on January 13, 1944, for observation. Obstipation was so severe that surgical consultants made a diagnosis of intestinal obstruction and were ready to do a colostomy. A Frei test done on January 14, 1944, was positive. On January 17, 1944, parahydrocyphenyl ethyl hexane, 30 mg. per day, was begun and continued until February 25, 1944, a period of five weeks. At this time the index finger could easily be passed through the area of previous stricture, and the patient stated that although she was more or less nauseated, she had daily formed stools. The medication was changed to hydroxyphenylhexane, 18 mg. per day, and continued for another two months, after which all medication was discontinued (April 28, 1944) because she was symptom-free and rectal examination revealed no stricture. On May 12, 1944, the sedimentation rate was 65 mm. in 45 minutes; Kahn and Kline were negative; and the total serum protein concentration was 8.2, the albumin, 3.8, the globulin, 4.4 grams per cent. Since this indicated persistent infection in spite of the fact that she was free from symptoms, sulfamerazine 3.8 gm. per day, was administered for seven days. The patient was not seen again for seven months during which time she had no medication.

On February 5, 1945 she returned in response to a letter, and stated that she was having normal, formed bowel movements daily. Upon examination the index finger passed the stricture area easily, although a non-constricting fibrous band was still palpable on the right rectal wall.

Case 2. Mrs. A. G., aged 56, a white woman of Puerto Rican origin, was first seen on February 28, 1944, complaining of abdominal cramps and distension with discomfort after eating. Her constipation was so severe that she had to resort to daily purgatives so as to maintain a liquid stool. In this manner she had from ten to twelve liquid stools per day. She also complained of passing stools through the vagina. All of these symptoms had existed for 12 years. About 5 years before we saw her she was a patient at

another hospital where a diagnosis of chronic ulcerative colitis with stricture of the rectum was made and a colostomy was advised. A biopsy of rectal mucosa at that time showed extensive acute and chronic inflammation and superficial ulceration. The Kahn, Kline and Wassermann tests were negative and x-ray of the colon following a barium enema showed no definite abnormality. A Frei test done April 21, 1944 was positive. Examination revealed a narrowing of the rectum just above the anal margin. A proctoscope could be passed to, but not through, the stricture. There was a copious discharge of pus, blood and mucous with evidence of ulcerative proctitis. A diagnosis of stricture of the rectum, ulcerative proctitis and lymphogranuloma venereum was made. The vaginal examination showed normal adnexae and uterus. However, methylene blue taken by mouth revealed a recto-vaginal fistula which extended from the posterior vaginal wall near the mucocutaneous junction into the rectum.

Treatment: Beginning on April 22, 1944 the patient was given parahydroxyphenyl ethyl hexane, 5 mg., three times a day, and on April 28, 1944, this was increased to 20 mg. a day. At this time she was also given Neoprontosil, 2 gm. per day, to help clear up the secondary proctitis. On May 1, 1944 she began to improve clinically and the stricture was gradually decreasing so that the rectum now almost admitted a finger. On May 5, the stricture had disappeared, except for a non-absorbed fibrous band. The Neoprontosil was discontinued but she continued to take parahydroxyphenyl ethyl hexane, 20 mg. per day. On May 12, 1944, the rectum admitted a finger, and the stools were well formed. On May 27, 1944, the parahydroxyphenyl ethyl hexane was reduced to 10 mg. per day, and on June 2nd it was further reduced to 5 mg. per day. The patient now felt well, had a normal stool and, except for the passage of stool through the vagina due to the recto-vaginal fistula, she was in good health. On June 6, 1944, her serum albumin-globulin ratio was reversed with an albumin of 3.3, globulin 7.8, total protein, 11.1 gm. per cent. On June 26, 1944 a course of sulfadiazine, 4 gm. per day for 8 days, was instituted and the estrogens were discontinued. On July 15, 1944, the serum albumin-globulin ratio was still reversed with an albumin of 1.8, globulin, 10, and total protein, 11.8 gm. per cent. The sedimentation rate was 75 mm. per hour. On October 2, the parahydroxyphenyl ethyl hexane therapy was again begun with 5 mg. every other day. The serum albumin-globulin ratio was still reversed; albumin, 4.6, globulin, 4.87 gm. per cent. The estrogens and sulfonamides did not change the albumin-globulin ratio or lower the sedimentation rate. The patient was last seen January 25, 1945, having had no medication for the previous two months. She had no rectal symptoms, was able to pass a wel-formed stool and was comfortable, except that she still passed stools and gas through the vagina by way of the recto-vaginal fistula.

Case 3. E. J., 45 years old, a para 1, was first seen in the proctological clinic on April 12, 1944, at which time a rectal stricture, annular in type, was found about 5 cm. up the canal. The stricture permitted passage only of a finger tip. The Frei test was positive. She had been having loose stools for several years. The sedimentation rate was 40 mm. in 45 minutes. Gynecological examination disclosed the uterus to be enlarged to the size of 12 to 14 weeks of gestation due to multiple fibroids. Although the periods were regular (every 26 days) the flow was profuse and lasted ten to twelve days.

Treatment and progress: On April 28, 1944, parahydroxyphenyl ethyl hexane, 30 mg. per day, was started. May 5, 1944 (one week later): "Stricture seemed to be relaxing. Patient stated her stools were now ribbonlike and partially formed for the first time." The same medication was continued. The serum albumin-globulin ratio was reversed. The patient did not return until July 14, 1944, and then only by written request. After finishing two weeks of therapy she took no treatment for two months. She stated that the stools had been normal and that the last two menstrual periods were only of seven instead of twelve days' duration. Examination demonstrated that the stricture area could be passed easily by the index finger. The fibroids seemed smaller in size. The patient was given ethinyl estradiol 0.1 mg. and estrone sulphate 2.5 mg. per day for two weeks only. When she returned two months later, September 15, 1944, a finger could be introduced into the rectum easily while pelvic examination showed that the uterine fibroids were smaller. Sulfaguanidine, 4.0 gm., was given daily for ten days. The serum albumin-globulin ratio was still reversed. No medication was given. Three weeks later, October 20, 1944, she was instructed to take estrone sulphate, 3.75 mg. per day. She came back a month later, November 17, 1944, claiming that she never felt better in her life. The stools were normal and hardly any evidence of stricture could be found. Menstrual periods occurred at 30 day intervals, and were of 6 to 7 days' duration. The uterus was only slightly enlarged. She refused further medication, believing it to be unnecessary. The patient has since moved and temporarily cannot be reached.

Case 4. S. B., 25 years old, was admitted to the Orthopedic Service of the Hospital for Joint Diseases on December 7, 1944, with a non-specific synovitis of the left knee of 18 years' duration. On December 18, 1944, the synovium and medial cartilage were excised. The pathologic diagnosis was chronic idiopathic synovitis. At this time it was noted that the patient had not moved her bowels. Mild cathartics caused no bowel action and strong cathartics produced a watery stool. The patient admitted having difficulty moving her bowels for several years and that the condition was getting worse, so that now she could have a movement only when

the stool was liquid. The Kahn and Kline tests were negative. The Frei test was positive. The sedimentation rate was 47 mm. in 45 minutes. On December 28, 1944, rectal examination revealed the presence of a stricture about 3 cm. from the anal orifice. This stricture was quite firm, and did not admit the tip of the examining finger. The serum albumin-globulin ratio was 4.1 to 4.0 gm. per cent. Sulfamerazine, 3.0 grams per day, was given beginning on January 2, 1945, and was continued for three days only. On January 5, 1945, estrone sulphate 3.75 mg. per day, and diethylhydroxymethoxystilbene, 1.5 mg. per day, was started and continued for a period of 33 days. On January 11, 1945, after 6 days of estrogen therapy, the patient stated that her bowel elimination was definitely improved. The stools were of soft, formed consistency. Side reactions of nausea and mild abdominal pains were noted. After 12 days of estrogen treatment, examination disclosed the stricture to be elastic in consistency. It was now possible to pass the tip of the index finger without difficulty. Bowel movements were formed and approximately of this calibre. After 17 days of estrogen therapy, the index finger could be passed through the stricture and the stools were almost normal. By the 33rd day of estrogen therapy, the daily stools were the calibre of the index finger.

CONCLUSIONS

1. Large doses of orally effective estrogens may allow sufficient dilatation of rectal strictures due to lymphogranuloma venereum to relieve the patient of symptoms of intestinal obstruction, and to render operative interference unnecessary.

2. The dose of estrogens necessary to produce the desired effect is apparently at least three to five times the average recommended therapeutic dose. We prefer daily oral administration of estrogens in order to maintain a continuous effect similar to that found in pregnancy.

3. The increased vascularity and softening of the rectal mucosa induced by estrogen therapy may be the mechanism by which dilatation of the stricture is produced.

4. The symptom-free state has persisted up to 7 months after discontinuation of estrogen treatment in two of the four cases.

5. The use of sulfonamide in addition to estrogen therapy has been of value only in clearing up the secondary proctitis.

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The Treatment of Angina Pectoris with Testosterone Propionate

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RECENT studies indicate that androgenic treatment may be of considerable therapeutic value in angina pectoris. This use of the male hormone has been reported to reduce the frequency and severity of attacks, to increase the exercise tolerance, and to produce a feeling of well-being. However, in some instances there was gradual recurrence of symptoms on discontinuation of treatment and in a few there was questionable improvement or none whatsoever. Table I summarizes these studies as recorded by the various investigators. The selection of cases and the criteria of improvement applied by these workers were not always beyond scientific criticism, results being appraised in most instances on the basis of subjective evidence. For instance, the daily nitro-glycerin requirement is an extremely variable factor, since attacks vary in severity and frequency from day to day and even during any given day. The two-step exercise tolerance test employed by Lesser (7) was the only approach to an objective evaluation under standard conditions.

In order to obtain a more objective method of determining response to treatment with testosterone propionate, a different attack was chosen in this study.

PROCEDURE

The general procedure called for careful selection of the patients and for objective assessment of the results of testosterone propionate treatment by means of exercise tolerance tests in conjunction with electrocardiographic studies both before initiation of therapy and at its termination.

Diagnosis and choice of patients. The diagnosis of angina pectoris was made on the basis of the history and the physical examination. Electrocardiograms were taken before and after exercise. It is known that in angina pectoris the electrocardiogram is frequently normal. However, 20–40 per cent of the patients who are suspected of having angina and who show a normal electrocardiogram will exhibit a positive electrocardiographic test to exercise, indicating the presence of coronary insufficiency. In order to eliminate the possibility of pseudo-angina or climacteric anginal syndrome, only those patients who responded with a positive test as described by Master *et al.* (11) were used for the purpose of this study.

The exercise test. The procedure devised by Master was followed. An electrocardiogram was taken prior to the test. With electrodes still in place, each patient ascended on one side and descended on the opposite side of two, nine-inch steps, then turned and repeated the procedure. The number of steps required for each individual was calculated according to prepared tables (11). The test was always performed about one hour after breakfast, at a room temperature of 68–78° F. The room was kept quiet and no other person allowed in it, in order to avoid any distracting influences. After exercise, the electrocardiogram was repeated. With sufficient experience, a five-lead electrocardiogram can be taken within 60 seconds after completion of the exercise. If the graph is recorded within a period of 2.5 to 3 minutes one can be quite certain that very few cases with positive deviations will be missed. The same time interval was used in all experiments in order to obtain uniformity and thus facilitate subsequent comparison studies. Normal contours usually reappear in about 8

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TABLE I. TESTOSTERONE PROPIONATE IN THE TREATMENT OF ANGINA PECTORIS AS RECORDED IN THE MEDICAL LITERATURE

Author	No. of Cases	Diagnostic Criteria	Dosage	Therapeutic Criteria of Improvement and Results	Proposed Mode of Action
Walker, T. C. (18)	5	Clinical evidence.	No comment.	In 4 cases increased emotional and exercise tolerance; decreased severity of pain; general increase in strength; no ECG findings stated.	No comment.
Lesser, M. A. (6)	24 20 M 4 F	Clinical evidence; ECG.	25 mg. every 2–5 days, totaling 5–25 injections (Average 11).	In all cases, lasting from 2 to 12 months after cessation of treatment, diminution in frequency, duration, and severity of attacks; increased tolerance for exercise; (Improvement noted after first injection in some) no effect on ECG.	Promotion of coronary collateral circulation.
Hamm, L. (4)	7	Clinical evidence; ECG.	25 mg. 3 times weekly for from 6 to 8 weeks, gradually reduced to 25 mg. once a month (Average no. of injections a yr.: 30).	In all cases, general improvement in mental and physical endurance. Symptom-free state lasting from 6 to 11.5 months. Improvement noted after average of 4 injections.	Vasodilatation and promotion of coronary collateral circulation; general tonic effect with improved feeling of well-being.
Walker, T. C. (19)	9	Substernal discomfort precipitated by exertion and relieved by rest.	4×25 mg. first week, gradually reduced to 10 mg. a week—12 weeks course.	In 7 cases increased tolerance to all precipitating factors; increased strength, decreased severity of pain in some.	Pronounced vasodilatation; probable psychotherapeutic effect.
Lesser, M. A. (7)	22 21 M 1 F	Clinical evidence; ECG.	25 mg. spaced according to response up to 25 injections.	In all cases diminution in frequency, duration, and severity of attacks; in 4 patients exercise tolerance test was performed, giving proof of increased tolerance for exercise and of shorter duration of pain.	No comment.
Sigler, L. H.; and Tulgan, J. (14)	20	Clinical evidence; hyperactive carotid sinus reflex; ECG (abnormal in 12)—history of coronary occlusion in 12.	25 mg. twice weekly.	In 11 cases marked relief (diminution in pain; increased tolerance for exercise; feeling of well-being). In 5 cases some relief. In 4 cases no relief. No changes in physical or ECG findings. Gradual recurrence of symptoms on discontinuation of treatment.	No comment.
Levine, S. A.; Likoff, W. B. (8)	19 16 M 3 F	Clinical evidence; ECG; history of coronary thrombosis in 3.	25 mg. 3 times weekly, for 4 weeks (In one patient for 7 weeks).	In 5 cases marked improvement (Increased tolerance for exercise; decreased requirement of nitroglycerin). In 1 case moderate improvement. In 2 cases questionable improvement. In 11 cases no improvement.	No comment.
Strong, G. F.; and Wallace, A. W. (15)	14	Clinical evidence; ECG in some cases.	25 mg. every 4–5 days, totaling generally 12 injections.	In 5 cases marked improvement (Increased tolerance for exercise; decreased requirement of nitroglycerin; feeling of well-being). In 7 cases slight to moderate improvement. In 2 cases no improvement.	General tonic effect with improved feeling of well-being. Vasodilatation or prevention of vasospasm.

minutes. It is important in performing this test that the string be accurately standardized, with one mv. producing a deviation of one cm., so that elevations or depressions in contour will not be magnified or minimized.

The criteria set forth by Master were used in corroborating the diagnosis of coronary insufficiency. In those patients with angina pectoris who react positively, exercise will cause characteristic alterations in the electrocardiogram, usually a depression of the ST segment, or T wave changes. In performing the test caution must be exercised since it is inadvisable to encourage such exertion on patients in whom occlusion may be precipitated. It is imperative to stop the test immediately if severe pain begins.

Control Period. Each patient served as his own control for a period of two months. During this time the usual treatment was employed (xanthines, vasodilators, sedatives, nitroglycerin, etc.). Electrocardiographic and exercise tests were taken at the onset in all cases but one, and at the termination of the two months' control period in all cases. During this time observations were made on the requirement of nitroglycerin, exercise tolerance, work capacity, physical and mental reactions; and the patient was interrogated as to the status of his libido. Electrocardiographic studies, before and after exercise, were thus obtained, both at the onset and at the termination of the control period.

Treatment. Following the observation period each patient was given intramuscularly 25 mg. of testosterone propionate[2] twice weekly for a period of eight weeks. In addition nitroglycerin was used whenever necessary to relieve attacks. At the end of this period of eight weeks the electrocardiographic studies were again repeated, before and after exercise under standard conditions. The degree to which testosterone propionate prevented electrocardiographic alterations in response to exercise, or a reduction in such alterations when present, was considered an objective index of the therapeutic effect. This is in agreement with Riseman's statement (13) that "most therapeutic measures that decrease myocardial anoxia and thereby enable the patient to do more work without pain, prevent or minimize the electrocardiographic changes that accompany exercise."

This procedure was followed in ten white male patients ranging in age from 43 to 58 years (average 50.1 years).

[2] I am indebted to Dr. Leo Pirk of Roche-Organon who generously supplied the testosterone propionate (*Neo-Hombreol*) used in this study.

CASE REPORTS

Case 1. R. M., aged 46 years. Since 1938 this patient had complained of vise-like midsternal pain, which radiated to both shoulders, and was brought on by exertion, cold showers, or emotional upset. These attacks were accompanied by palpitation and slight dyspnea at the height of the pain. Definite and immediate relief followed the sublingual administration of a nitroglycerin tablet (1/150 grain), of which eight to ten were taken daily. Delayed relief followed rest. Electrocardiograms made in 1940 and 1942 were described as normal.

Examination in October, 1943 revealed a blood pressure of 120/84. There was no evidence of cardiac enlargement, no murmurs, and the tones were of fair quality. The pulmonic second sound was louder than the aortic second sound. Fluoroscopy revealed no enlargement of the chambers. The transverse diameter by the rapid method (11) was 135 mm., which was normal for an individual of this height and weight. The electrocardiogram at this time, the beginning of the control period, showed ST_3 slightly elevated before exercise (Fig. 1A). Following exercise ST_1 was depressed 1 mm.; ST_2 was depressed 3 mm.; ST_3 was concave downward and depressed 1 mm.; T_3 was diphasic; ST in CF_2 became depressed 1 mm.; and the T-wave in this lead became peaked and increased in voltage. ST in CF_4 was depressed 2 mm. and the T-wave was high and peaked (Fig. 1B). At the end of the control period no unusual changes from these findings were noted, despite the treatment with aminophylline, papaverine, and other coronary vasodilators. The symptoms were practically unchanged.

The patient was then started on the injections of 25 mg. testosterone propionate twice weekly. Relief was noted after the third injection at which time only two to three nitroglycerin tablets were needed daily. After the tenth injection only one tablet was necessary daily. During the next week the patient suffered an upper respiratory infection and the dosage of the nitroglycerin tablets had to be increased to three or four daily. Following the 16th injection the patient felt well enough to return to work as a postal clerk. At this time the electrocardiogram was again repeated to determine what, if any, effect had been

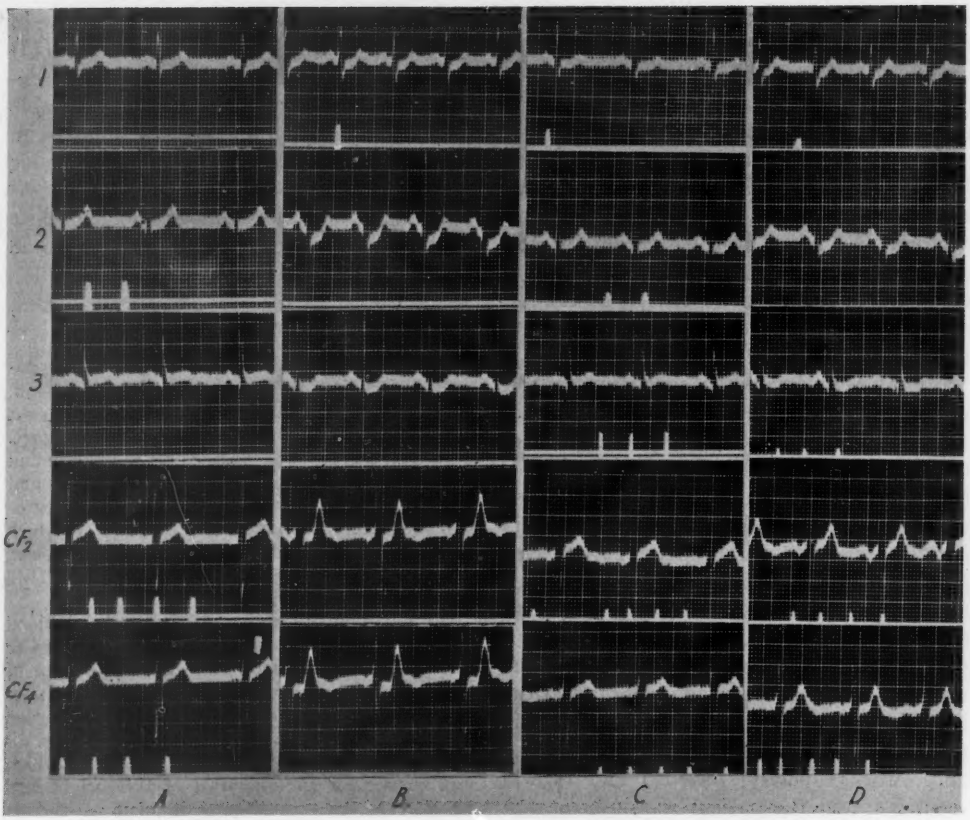

FIG. 1. Electrocardiograms of *case 1* (*R.M.*) Two months before institution of testosterone propionate treatment: A—Pre-exercise tracing; B—Post-exercise tracing. Eight weeks after institution of testosterone propionate treatment: C—Pre-exercise tracing; D—Post-exercise tracing.

brought about by the treatment. The pre-exercise electrocardiogram (Fig. 10) revealed no change from the pretreatment graph. However, the electrocardiogram taken after exercise was altered. Improvement was evident. ST_1 was still depressed 1 mm.; ST_2 was depressed only 2 mm.; ST_3 became less concave and was depressed only 0.25 mm.; ST in CF_2 was unchanged; and T increased to a lesser voltage. ST in CF_4 was now not depressed, and T was less (Fig. 1D). These findings showed less marked positive changes as compared to the pre-treatment post-exercise electrocardiogram. However, there was no change in cardiac outline or blood pressure. The patient noted a mild increase in libido, a general improvement in sense of well being, and was able to accomplish more in spite of the reduction in the dosage of nitroglycerin, which averaged one tablet daily.

The injections were discontinued for one month, when an increase in the pain and in the frequency of attacks were noted. Injections were then resumed at intervals of two weeks and gradually extended to an interval of four weeks, with a return of relief as previously noted. However, it must be stated that because of the rapid return of symptoms, when the patient received no treatment, a supplement of oral methyl testosterone was also given in doses of 10 mg. three times weekly.

Eleven months have elapsed since treatment was begun. The patient takes about three nitroglycerin tablets weekly, is active at his job, even to working on the night shift. Relief is sustained as long as treatment with methyl testosterone is continued.

Case 2. B. H., aged 48 years. For the past ten or eleven years this patient has complained of heaviness in his chest, the sensation radiating to the left arm and elbow, and these attacks occurred quite regularly following exertion. A posterior myocardial infarction was sustained in 1938 after which there was an increase in the frequency of attacks. These, however, were regularly relieved by the use of nitroglycerin

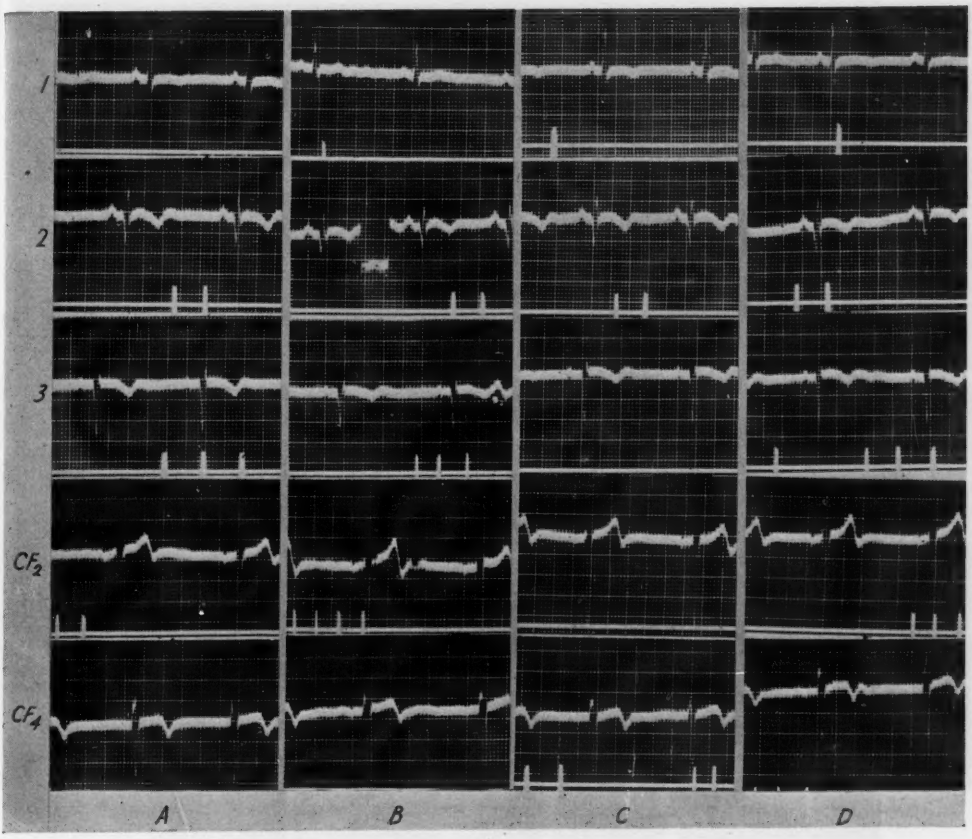

FIG. 2. Electrocardiograms of *case 2* (*B.H.*). Two months before institution of testosterone propionate treatment: A—Pre-exercise tracing; B—Post-exercise tracing. Eight weeks after institution of testosterone propionate treatment: C—Pre-exercise tracing; D—Post-exercise tracing.

tablets, of which from four to seven were taken daily. Mild exertion caused slight pain for which rest proved a satisfactory antidote.

Examination revealed a blood pressure of 180/90. There was no cardiac enlargement, no murmurs were heard, and the heart's tones were of good quality. An electrocardiogram in 1938 showed posterior myocardial infarction. In December 1943 an electrocardiogram revealed a persistent Q_3T_3 pattern. In March, 1944, an electrocardiogram was taken during an exercise test. The pre-exercise tracing showed no change from the graph taken in 1943. The Q_3T_3 pattern was evident with T_1 and T_2 inverted. In the chest leads, T was diphasic (Fig. 2A). Following exercise, (Fig. 2B) ST_1 was depressed, T_1 became upright, T_2 was diminished, T in CF_2 became more diphasic and increased in voltage. After a two months' control period with the usual medication, no changes were evident either in the pre- or the post-exercise electrocardiogram. The general condition and attacks varied with the usual vacillations occurring in the anginal syndrome.

Testosterone propionate was then started in dosage of 25 mg. semiweekly. Following the 12th injection the patient observed marked improvement, with his pains disappearing completely. No nitroglycerin was necessary, and the general well being showed marked improvement to the extent that the patient began to overexert as compared with his earlier activity. Following the 16th injection, the electrocardiogram before exercise showed a slight increase in the T-wave in lead CF_2 (Fig. 2C). After exercise the tracing showed a response similar to that of two months earlier, except that the T in CF_2 showed less of a higher voltage response and less of a diphasic swing (Fig. 2D). These findings may be interpreted as indicating some improvement in the post-exercise electrocardiogram.

No further injections were given and the patient was well in November 1944. There was no change in libido.

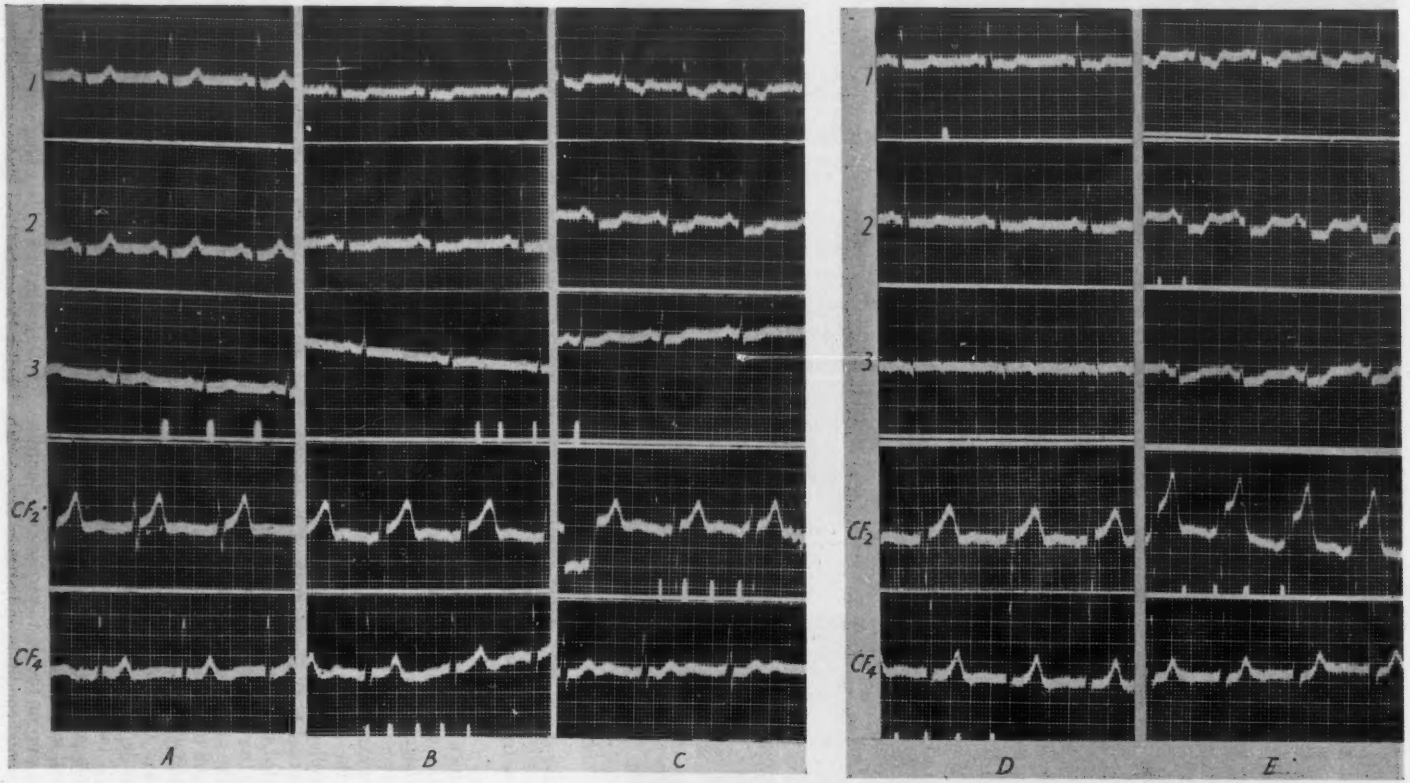

FIG. 3. Electrocardiograms of *case 3* (*W. B.*). Four months before androgen therapy: A—Pre-exercise tracing. Two months before institution of testosterone propionate treatment: B—Pre-exercise tracing; C—Post-exercise tracing. Eight weeks after institution of testosterone propionate treatment: D—Pre-exercise tracing; E—Post-exercise tracing.

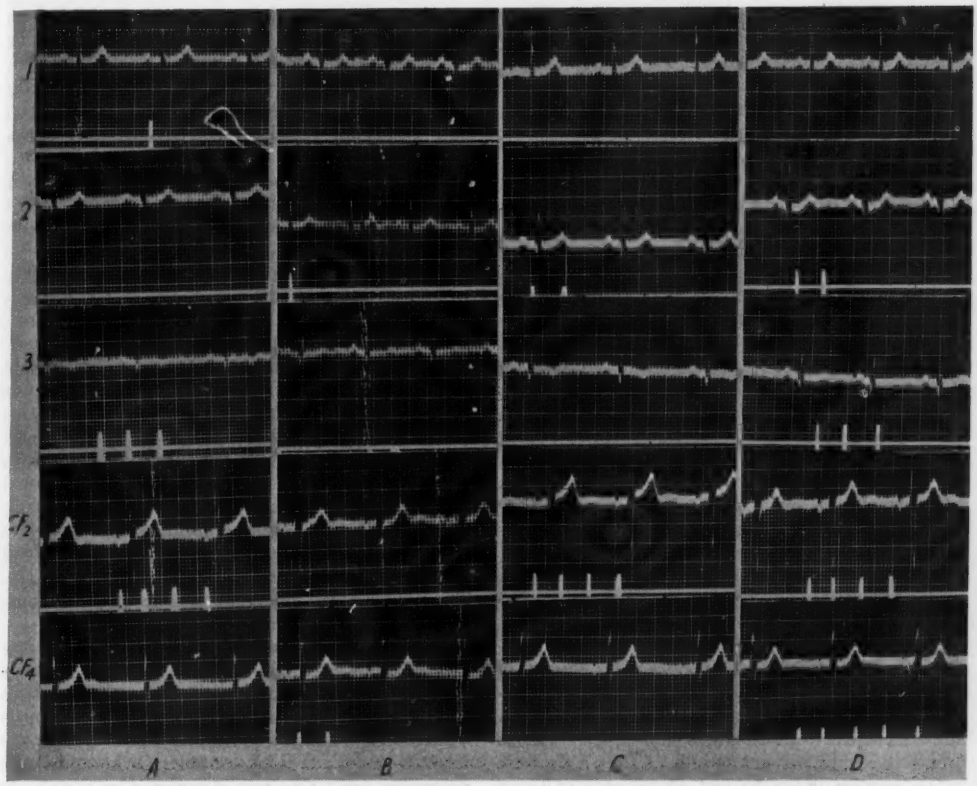

FIG. 4. Electrocardiograms of *case 4* (*W.S.*). Two months before institution of testosterone propionate treatment: A—Pre-exercise tracing; B—Post-exercise tracing. Eight weeks after institution of testosterone propionate treatment: C—Pre-exercise tracing; D—Post-exercise tracing.

Case 3. W. B., aged 58 years. Precordial pain radiating to the left arm had been present for four months; it was brought on by walking as little as one block. At the same time there was pain in the calf of the left leg occurring after exertion. Both symptoms were relieved by rest. The cardiac pain promptly responded to the sublingual administration of nitroglycerin tablets of which from four to eight were taken daily.

Examination revealed the blood pressure was 136/74; the pulse was 80; there was a slight enlargement of the heart whose sounds were distant. No murmurs were heard. The oscillometric reading of the left leg above the ankle was 0.75; and on the right, in a similar position, it was 2.50. An electrocardiogram, which had been made about two months earlier, showed no abnormalities (Fig. 3A). No exercise test had been made at that time. Various treatments and injections including *Depropanex* were not attended by improvement. An electrocardiogram was then made which revealed (Fig. 3B) before exercise definite alterations in the ST and T waves, indicating coronary changes. These findings were not evident two months before this. ST_1 was depressed 0.25 mm. with a diphasic T_1; ST_2 was depressed 0.5 mm. with diphasic T_2; T_3 was diphasic; and ST in CF_2 was elevated.

Following exercise there was definite evidence of coronary insufficiency. The QRS in lead 1 became more slurred; ST_1 was depressed 1 mm. with an inverted T_1; ST_2 was depressed 2 mm., with a diphasic T_2; ST_3 was depressed 0.5 mm.; ST in CF_2 became flattened; and T in CF_4 diminished in voltage and became slightly diphasic (Fig. 3C).

The patient was then given treatment with testosterone propionate in doses of 25 mg. twice weekly for 8 weeks. During this time there was no evidence of any improvement either in the precordial pain or in the intermittent claudication. After this treatment the electrocardiogram revealed further changes in the pre-exercise test. ST_1 became depressed 0.5 mm.; ST_2 was depressed 1 mm.; QRS_3 diminished in voltage

and a Q-wave appeared; and T_3 became inverted (Fig. 3D). Following exercise there was a greater response than before the treatment. ST_1 became depressed 1.25 mm.; ST_2 was depressed 3 mm.; ST_3 was depressed 2 mm.; ST in CF_2 became elevated 6.0 mm.; and ST in CF_4 remained the same (Fig. 3E).

The patient's nitroglycerin requirement was unchanged and there was no improvement in any way. It is apparent that this patient's coronary insufficiency was progressive before and throughout the treatment with testosterone. The electrocardiographic response to exercise showed even more marked evidence of coronary insufficiency. Treatment was discontinued. No change in libido was noted.

Case 4. W. S., aged 57 years. This patient had a sudden onset of localized, precordial pain in February, 1939. After that time he experienced recurrences on exertion that involved walking four or five blocks at his usual pace, or on awakening in the morning. Nitroglycerin relieved these attacks. An electrocardiogram made at that time was normal.

In December, 1943 the pain had increased in severity, duration, and frequency. An electrocardiogram at this time revealed the following changes: T_3 had become sharply inverted; QRS became W-shaped with a prominent Q-wave. This alteration was interpreted as an occlusion in a posterior coronary vessel with some myocardial infarction. Subsequent electrocardiograms showed spontaneous progressive improvement as indicated by a decrease in T_3, and an inversion and prominence of Q_3; a stationary graph was evident from September, 1943, to May, 1944. During this time the patient had felt fairly well, except for attacks of pain which were regularly relieved by the use of nitroglycerin.

In May, 1944, an examination revealed the blood pressure was 134/84, and there was some cardiac enlargement; the transverse diameter measured 165 mm. by the rapid method. The heart sounds were of good quality. The pulmonic second sound was greater in intensity than the aortic. The pre-exercise electrocardiogram showed no change from the previous graph. A Q_3T_3 pattern was still present (Fig. 4A). Following exercise, ST_1 was found to be depressed 0.5 mm., ST_2, 1 mm., ST in CF_2, 1 mm., and ST in CF_4, 1 mm. (Fig. 4B). These findings indicate a positive test.

During a two months' observation period no unusual changes were noted, and the electrocardiogram remained unchanged before and after exercise. Treatment with intramuscular injections of testosterone propionate was then begun. Following the fourth injection the precordial attacks of pain disappeared. After the full course lasting eight weeks there was marked clinical improvement. No nitroglycerin was necessary, although the patient always carried the tablets with him. He worked eight hours or more daily, had greater physical capacity, and he noted a slight increase in libido. The electrocardiogram taken after these treatments revealed QRS_3 was more upright than in the pre-exercise graph (Fig. 4C). Following exercise ST_1 remained isoelectric, ST_2 depressed 0.5 mm.; ST in CF_2 depressed 1 mm.; and ST in CF_4 remained isoelectric (Fig. 4D). A moderate decrease in the post-exercise alterations was evident, indicating improvement. No further injections were given, and relief is continuing. No change in blood pressure was noted.

Case 5. R. M., aged 43 years. Pre-sternal oppression had been present since September 1941, recurring regularly following exertion or excitement. He was particularly worrisome since several of his friends had recently died of coronary thrombosis. His pains were relieved by the sublingual use of nitroglycerin tablets, each 1/200 grain. An electrocardiogram in November, 1943 was normal except for occasional ventricular premature systoles. The patient's condition remained the same—he needed from two to five nitroglycerin tablets during 24 hours. The blood pressure was maintained at approximately 152/96. The heart was not enlarged, and there were no murmurs heard.

In January, 1944, another electrocardiogram was taken. The pre-exercise graph showed the following changes when compared with the graph taken in November, 1943: T_2 became flattened from a previous voltage of 3 mm.; T_3 became diphasic, a change from its earlier upright contour; Q_3 became more prominent; and CF_4 was diminished in voltage to 2 mv. from a previous 5 mv. After exercise, a positive test for coronary insufficiency was obtained, viz.: ST_1 became depressed 1 mm.; T_1 was diphasic; ST_2 was depressed 2 mm.; and T_2 was diphasic; ST_3 was depressed 0.25 mm., and T_3 became inverted; ST in CF_2 was elevated 2 mm., and the T in this lead became peaked; T in CF_4 became diphasic.

After two months' observation period, the initial pre-exercise test showed a further inversion of T_3 from a previous diphasic contour. The post-exercise test showed further depression in ST in leads 1 and 2, with increased peaking of T in CF_2. Semi-weekly injections of 25 mg. of testosterone propionate were then given for eight weeks. The patient felt better, but noted no diminution in the frequency, severity and duration of the attacks of precordial pain. No change in the nitroglycerin requirement was noted. Exercise tolerance, libido, and blood pressure remained about the same. The electrocardiogram at this time showed further progressive changes in the pre-exercise test with further inversion of T_1, T_2, and T in CF_4, indicating a progression of involvement of the anterior coronary vessel. After exercise the electrocardiogram showed sharper inversion in T in leads 1, 2, 3, and CF_4. Injections were discon-

tinued. It should be noted that this patient's condition was becoming progressively worse before treatment with testosterone was instituted.

Case 6. B. H., aged 51 years. Since 1940 anginal pain with typical radiation to the left arm had been present. Four to six tablets of nitroglycerin were needed to relieve the pain which would follow fast walking, emotional disturbances, and hearty eating.

Physical examination revealed hypertension (blood pressure was 170/110), enlargement of the heart (measuring 165 mm. by the rapid method), an apical systolic murmur, but good heart sounds. The basal metabolic rate was plus 6.

An electrocardiogram taken at the onset of the observation period, January, 1943, revealed only a slight slurring of QRS in leads 1 and 3. The exercise test caused a depression in ST_1 of 0.5 mm., and in ST_2 of 1 mm.; T_2 became diphasic; ST in CF_4 was depressed 1 mm.

For two months this patient was treated with theobromine and its derivatives, with papaverine, and with sedatives and no marked change in symptoms occurred. He continued to take nitroglycerin as before, and his electrocardiogram remained the same. After seven injections of testosterone propionate, 25 mg. each, he began to note some relief. Following the sixteenth injection, the patient found it necessary to take but one nitroglycerin tablet daily. Other attacks which occurred during the day were too mild to warrant taking the medication. The heart did not change in size. The blood pressure remained elevated at 168/102. The electrocardiogram at this time showed some increase in the voltage of QRS but no other changes were manifest in pre- or post-exercise electrocardiograms. No further injections have been given in 18 months, and his condition remains the same. No increase in libido became apparent.

Case 7. K. T., aged 48 years. This man had had an attack of coronary occlusion about 5 months prior to this study. Substernal pain and pressure would appear on walking from one and a half to three blocks. In the preceding three months, the pains had increased in severity, but were relieved by the administration of nitroglycerin, of which from six to twelve tablets were used daily. The electrocardiogram taken three months after the occlusion still showed a Q_3T_3 pattern.

Examination during November, 1943 revealed an obese man weighing 200 lbs.; his blood pressure was 130/80. The heart could not be outlined by percussion but did not appear enlarged on fluoroscopy. No murmurs could be heard, but the sounds were very distant. The basal metabolic rate was found to be plus 12. An electrocardiogram showed a blunt T_1, small Q_3, and inverted T_3; T_4 was diphasic. This showed improvement over the electrocardiogram taken 3 months before. After exercise, T_1 became inverted, ST in CF_4 was depressed 2 mm., and T in CF_4 was inverted. After 2 months with the usual treatment, no change was noted in the symptomatology or in the electrocardiograms taken before or after exercise.

Androgenic therapy was instituted and following the 12th injection of testosterone propionate, marked clinical improvement was noted. The patient felt generally better, required only from two to three nitroglycerin tablets daily, and went back to work as a bartender, although this was not advised. After 16 injections examination revealed no alterations in the findings with regard to the heart or the blood pressure. There was no change in libido. The electrocardiogram showed no change in the pre-exercise test. After exercise T_1 showed a diphasic rather than an inverted contour; otherwise the graph was as before treatment. No treatment other than the nitroglycerin was taken for six months, when symptoms began to recur (July, 1944). Another series of 16 injections relieved the patient.

Case 8. M. J., aged 51 years. The patient complained of pain in precordium radiating down the left arm and occurring after moderate exertion, such as walking from three to four blocks, or after unusually hearty meals. This condition had its onset seven years ago, and was punctuated by a myocardial infarction two years after its onset. Of late he noted a progressive decrease in the amount of exertion required to produce pain. From seven to twelve nitroglycerin tablets daily provided relief.

The examination disclosed an enlarged heart, poor heart sounds, no murmurs, and an accentuation of the pulmonic sounds. The blood pressure was 142/82. Fluoroscopy revealed a markedly enlarged left ventricle, and the heart measured 200 mm. in the transverse diameter (the rapid method). The electrocardiogram showed a prolongation of the A-V conduction time (PR equals 0.28 seconds), an intraventricular conduction defect (QRS equals 0.17 seconds), and marked left ventricular preponderance. The electrocardiogram never had approached normal after the occlusion, and the damage had appeared as a permanent record on the electrocardiographic tracings during the previous four years. Following the exercise test ST_2 and ST_3 became depressed; T_2 was diphasic; and T_3 became inverted. During the two months' control period, no changes were noted in his general condition, and no change was evident in the pre- or post-exercise electrocardiogram. While the patient received the injections of testosterone propionate, he noted periodic intervals of mild relief which was not more marked, however, than that which was experienced before the institution of hormonal treatment. After the fourth injection his nitroglycerin requirement was reduced to from five to eight tablets daily, only to return to his previous requirements shortly thereafter. For one or

two days after an injection the patient thought he felt better but his general condition and the frequency of attacks disproved this impression. After the full series of 16 injections the patient felt no better than before, and no significant changes were noted in heart, blood pressure, or electrocardiogram before and after exercise. He required nitroglycerin following each exercise test. No change in libido was noted. Because of the poor results, treatment with testosterone was discontinued.

Case 9. L. H., aged 53 years. The patient has had precordial pain radiating into the left arm and elbow for the past two years following exertion such as was caused by walking five or six short blocks or by playing six holes of golf. These attacks were relieved by nitroglycerin of which only one or two tablets were taken daily. In March, 1944, the blood pressure was 138/86. No enlargement of the heart was present, but soft systolic murmurs could be heard at the apex and in the aortic area. The aortic second sound was accentuated. The electrocardiogram was normal before exercise, but depressions in ST_2 and ST in CF_2, with a diphasic T_2 followed exercise. After a two months' control period no changes were noted. Following the fourth injection of testosterone propionate, relief was so marked that no nitroglycerin was required. After completion of the course of 16 injections, the patient felt well, needed no nitroglycerin, was able to do more physical work than before treatment with testosterone, and in general was quite happy. After exercise the electrocardiogram showed a less marked depression of ST_2, but no change appeared in CF_2 as compared with the previous post-exercise graph. When last seen six months after termination of treatment, the patient was still enjoying symptomatic relief. No change in sexual activity was noted.

Case 10. H. A., aged 46 years. The patient complained of substernal pain on exertion after walking from one to three blocks, or after climbing one flight of stairs. This symptom was only of three months' duration and was relieved by nitroglycerin of which he used from three to four tablets daily. Examination revealed no cardiac enlargement, no murmurs, good tones, and normal fluoroscopic contours. Blood pressure was 122/78. The electrocardiogram at the beginning of the control period (Sept., 1943) showed a small Q_3, a moderate Q_3, and an isolectric T_3. Following exercise ST_1 became depressed 2 mm., ST_2 remained isoelectric, ST_3 became elevated 1 mm., and T_3 was inverted so that a Q_3T_3 pattern appeared. Two months later under usual anginal treatment no change was noted in the electrocardiogram, nor was there any marked change in symptoms. Treatment with testosterone propionate was then begun. After the fourth injection the patient claimed marked relief and did not require more than one nitroglycerin tablet per day. Other attacks were "too mild to take the dynamite." After the full series of 16 injections no nitroglycerin was necessary. There was marked general improvement, increase in exercise tolerance, slight increase in libido, but no significant change in blood pressure. The pre-exercise electrocardiogram showed no change, but following exercise T_3 did not become inverted, but was diphasic. Other post-exercise alterations were as before. Six weeks following discontinuation of hormonal therapy some pain recurred. Consequently the patient was given one injection of 25 mg. testosterone propionate weekly for four weeks and thereafter once a month; this latter dose is still being given.

DISCUSSION

Although the series of cases presented here is small, the different method of approach to the study of the effect of testosterone in angina, by means of repeated electrocardiographic tracings with and without exercise, seems to have brought out some findings which on further study may prove conclusive.

Of the ten patients studied, seven showed definite improvement following treatment with testosterone propionate. These results by and large are in agreement with those observed by other investigators. Eight of the ten had stationary electrocardiograms for at least two months before treatment with testosterone was instituted. Of these eight patients, seven were improved and one failed to respond favorably to androgenic therapy. The latter patient had suffered a previous coronary occlusion and exhibited marked myocardial damage, with prolonged A-V conduction time and intraventricular block. Three of the patients who experienced improvement had also had a previous occlusion. The remaining two patients of the group of three who failed to improve had one thing in common. Both had progressive changes in the electrocardiogram before treatment with testosterone was started and both reacted poorly to treatment; in fact, this was discontinued after the first course. Of the seven improved cases, six showed some electrocardiographic evidence of improvement after exercise.

It is thus evident that patients whose electrocardiograms during the premedication control periods were stationary were benefited by treatment with testosterone propionate, while those with progressive changes indicating a progressive lesion, failed to improve. In other words, testosterone propion-

ate probably is of value in those cases where the pathologic changes in the coronary arteries, as revealed by electrocardiographic studies are at a standstill; the improvement brought about by the testosterone is sufficient to manifest itself clinically by symptomatic improvement, and frequently by improvement in the electrocardiogram taken after a calculated amount of exercise. On the other hand, if the pathologic changes are progressive, testosterone propionate will not arrest the progress of the lesion and therefore is of no value. These findings suggest a method of selecting prospective candidates for treatment: those patients who over a period of two or more months show stationary electrocardiograms before exercise, will probably respond favorable, while those who show continued progressive changes will probably fail to respond.

The number of injections of testosterone propionate required for relief has varied with different investigators. In our ten cases improvement was claimed by the respective patients following from three to twelve injections with an average of about six. Two patients are under a continuous form of treatment. One patient required two courses of sixteen injections each, and four patients obtained sustained relief on one course. It appears that a minimum of sixteen semiweekly injections of testosterone propionate 25 mg. each, would be advisable. After this course, individualization is necessary, depending upon the response of the patient. Lesser (7) has also stressed the importance of individualizing treatment. Treatment with testosterone is of no value in the acute attack of angina pectoris when, of course, nitroglycerin is indicated. Testosterone is to be employed as a means of preventing attacks or of reducing their frequency or severity, and is a form of treatment to be used over a relatively long period of time.

The possibility of undue stimulation of libido through testosterone administration with dire results for the cardiac patient was considered by Sigler and Tulgan (14). Only three patients in this series noted any increase in sexual desire.

CONSIDERATIONS OF THE PHYSIOLOGIC-PHARMACOLOGIC ACTION OF TESTOSTERONE PROPIONATE IN THE ANGINA PECTORIS OF CORONARY INSUFFICIENCY

Four possible actions of testosterone propionate have been suggested.

1. Vasodilation. In 1939, Edwards, Hamilton and Duntley (2) showed by spectrophotometric studies that the diminished arterial blood supply to the skin of castrated men was increased after the administration of testosterone propionate. On the basis of these observations they treated seven male patients with organic vascular disease by means of testosterone propionate. Included among these were three cases of Buerger's disease and four arteriosclerotics. All were improved. Herrmann and McGrath (5) subsequently observed similar results with estrogenic therapy in the vasospasm of angiitis. Several investigators (1, 4, 19) hold the view that vasodilation is the chief mode of action of testosterone propionate in angina pectoris.

2. Development of collateral circulation. Lesser (6) cites other investigators whose findings would indicate that adequate circulation may develop collaterally following partial or complete occlusion of one or more larger coronary arteries. At first sight, this mode of action seems unlikely since several patients felt improved after two or three injections of testosterone propionate. However, it has been reported (3), that intercoronary collateral development has been demonstrated experimentally to increase within a few hours and markedly increase in a few days, so that a preocclusion rate of flow may be attained. This was shown by studying retrograde flow in a severed main coronary artery. These findings seem to constitute substantiating evidence to this possible mode of action. However, if this were the only factor to be considered, one would expect results to be more lasting in the absence of progressive coronary changes in the other vessels. Many patients have been reported improved for a while, only to require a second

course of treatment later upon recurrence of symptoms.

3. Correction of androgenic deficiency. This mode of action is not too rational since angina pectoris is frequently unassociated with climacteric symptoms. However, it cannot be denied that in some cases, "a deficiency in gonadal hormone or an endocrine imbalance in certain individuals possessing an autonomic nervous system capable of an abnormal response is the factor which leads to functional or organic changes in the vascular system during or after" the climacterium (Bonnell *et al.* (1)).

4. Metabolic changes. McGavack (16) mentioned that "certain metabolic effects of a general character may play more than a cursory role in the action of testosterone propionate on the circulation." Indeed, recent biochemical studies and considerations of myocardial chemistry give some support to this view. It has been shown by Thorn and Engel (16) that in rats, castration is followed by a fall in the creatine phosphate and glycogen content of the heart. Testosterone administration restores these compounds to normal or above, and decreased the renal excretion of inorganic phosphorus.

Physiologically it is well established that phosphocreatine and adenosine triphosphate are intermediaries in the transfer of the chemical into the mechanical energy of the heart beat. These compounds are also essential to the oxidation of glucose and lactic acid in muscle metabolism. Myers (12) has studied the concentration of phosphocreatine and adenosine triphosphate in the human myocardium and found it to be an index of the potential capacity of the heart for work. It was discovered that where there was evidence of myocardial insufficiency or marked hypertrophy the involved ventricle showed a decrease from normal in the content of creatine, adenine, and phosphorus.

Although studies were not made in hearts with coronary artery disease, it is conceivable that an improvement in cardiac function or a correction of cardiac muscle metabolism may be effected by the action of testosterone propionate. In the light of the foregoing, this improvement may be predicated on better utilization of available oxygen. Gregg and Shipley (3) have recently emphasized the importance of changes in cardiac metabolism as a regulator of coronary flow. It seems possible that a local increase in metabolites is responsible for coronary dilatation through local action on the coronary vessels. Yet, according to Lewis (9) the pain in angina pectoris is believed to be due to stimulation of afferent nerve endings in the myocardium by an excess accumulation of metabolic products indirectly resulting from the oxygen insufficiency. It may thus be postulated that a small accumulation of metabolites is beneficial, causing vasodilatation of the coronary arteries, while large accumulations may cause pain due to the stimulation of sensory nerves. Testosterone propionate may mediate its benefits in part, through the correction and regulation of cardiac metabolism, permitting more muscular exertion before sufficient metabolites accumulate to produce pain. It is significant to note that in all cases heretofore reported, no evidence of cardiac failure has been mentioned as occurring in any patient under testosterone treatment and this may indicate a preventive effect.

SUMMARY AND CONCLUSIONS

A review of previous reports regarding the treatment of angina pectoris with testosterone propionate is presented in tabulated form.

Ten male patients with angina pectoris of coronary insufficiency, proven by electrocardiographic response to exercise, were treated with testosterone propionate. Each patient served as his own control during a period of two months under usual medicinal treatment. In eight patients no changes other than would be expected ordinarily occurred. Two patients showed evidence of progressively increasing coronary insufficiency during this time. Of the former group of eight patients, seven responded favorably to treatment with testosterone propionate while one failed to respond. Six of the improved cases also showed electrocardiographic evidence of favorable response. The two patients who

exhibited progressive changes during the control period failed to improve.

It is concluded that patients with stationary electrocardiograms are more prone to respond to treatment with testosterone propionate than those with evidence of progressively increasing coronary insufficiency.

An initial eight weeks' course consisting of 25 mg. of testosterone propionate twice weekly by intramuscular injection probably represents the minimum requirement for a sustained effect. Further treatment will vary with the individual case.

Since libido is very little affected, if at all, no undue stimulation with potential disastrous effects is to be expected.

A metabolic action of testosterone propionate through its action in increasing the phosphorus and creatine content of the heart muscle is postulated.

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EDITORIAL

This issue contains a letter which deserves the special attention of our readers, not only because it contains interesting material, but also because it is the first of a series that serves a special function this Journal's Editorial Board wishes to encourage. It is believed that our readers would enjoy and gain from a "ventilation" of interesting ideas, or criticisms, without the necessity of these remarks being highly documented or held in cautious reserve until every statement is finally proved. The Editors reserve the right to refrain from publishing contributions that seem unprofitably controversial. An opportunity would be afforded to reply to letters of general interest. It is hoped that this section will be used frequently.

LETTER

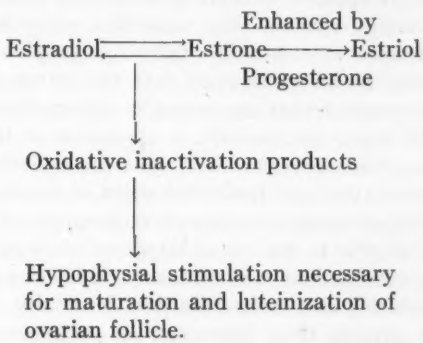
FUNCTIONAL FLOWING

To the Editor:

That a diagnosis of functional flowing is made in about 15 per cent of a gynecologist's patients seems less remarkable than that the incidence of this disorder is not greater. When one considers how complex and delicately balanced are the local and systemic changes of which endometrial bleeding is a manifestation, one cannot but wonder why those changes are not more often thrown out of rhythm by the many and varied insults, meteorological, physical, chemical, bacterial, dietary and emotional, to which womankind's "homeostasis" is continually being exposed.

Though the primary causes (with the exception perhaps of an intrinsic decrease of ovarian responsiveness at the climacteric) and intermediate mechanisms of functional flowing are still not clear, a deficiency of normal cyclic luteal activity is the obvious final cause in the typical patient in which non-bleeding and bleeding phases depend respectively on unregulated rises and drops of the estrogen level, while constant bleeding is presumably due to a teetering critical level. There seems to be a vicious circle of incomplete hypophysial-ovarian interaction, vicious and incomplete apparently because of the absence of the controlling and complementary influence exerted by the corpus luteum. A deficiency of progesterone may be taken as circumstantial evidence that estrogen is also deficient, as indicated by some endometria and the common occurrence of functional bleeding during the climacteric. Endometrial hyperplasia may thus be attributed to a prolonged action of subnormal amounts of estrogen.

Despite the lack of positive information, it is reasonable to postulate that patients with premenstrual staining or excessive flowing from a secretory endometrium also have deficient luteal secretion. This assumption is based on a clearer understanding of the mechanism of the luteal influence that has evolved from studies of estrogen metabolism in women. According to our present lights, products of the oxidation of extradiol and estrone set into play the hypophysial factors necessary for the corpus luteum's development and function, and progesterone in turn inhibits the formation of those products by speeding the conversion of estradiol and estrone to estriol. Graphically the situation is represented in the accompanying diagram.



Thus the corpus brings about its own regression. If it does not get optimum stimulation it will fail to suppress sufficiently the oxidation of estradiol and estrone and will continue to receive some stimulation, the result being incomplete regression and a critical level of progesterone, allowing partial and prolonged breakdown of the endometrium instead of the normal more or less "explosive" shedding of this tissue. This is the status, with or without abnormal bleeding, with which one associates premenstrual tension.

Experimentally, progesterone blocks ovarian stimulation by the anterior lobe; it puts the ovaries at rest. Clinical and pathological observation indicates that the ovaries remain *in statu quo* while the corpus luteum blooms. In addition to suppressing the factors which stimulate LH and luteotrophin, progesterone

apparently holds in check the ovarian response to FSH, and, furthermore, in physiological amounts, accomplishes these results without "doing anything" to the anterior lobe so far as can be determined by morphology or by the assay of transplants. With a drop in the level of progesterone, FSH is allowed to act, estradiol and estrone are produced, and there is a surge of the products of their oxidation resulting in the LH, which is so necessary for follicle maturation, ovulation and luteinization.

Experimentally, estradiol, estrone and diethylstilbestrol, in contrast with progesterone, cause not only morphological changes in the hypophysis but also a release of LH. Diethylbestrol apparently accomplishes these effects directly; whereas the naturally occurring estrogens must first be oxidized. The opinion seems to be widespread that the estrogens and progesterone are mutually antagonistic. The word, antagonistic, is applicable in the sense that progesterone inhibits the oxidative inactivation and hence the effect of secreted estrogen on the pituitary. It is also applicable in relation to the use of large and unphysiological amounts, which cause an inhibition of ovulation and of luteinization. Normally, in all respects these hormones are complementary and mutually interdependent. They are complementary as regards their effect on "end-tissues," *e.g.*, breasts and genitalia. Progesterone depends for its existence on the estrogen that precedes it. Indeed, it is likely that the respite afforded the anterior lobe and the unluteinized ovarian tissue during the luteal phase is necessary for the proper production of estradiol in the ensuing cycle. Even the adrenal cortex may be resting during the luteal phase.

It is now a truism that the endometrium, regardless of its state of proliferative or secretory development, is exquisitely sensitive to fluctuations of the quantity of ovarian hormones in the blood. Growth or regression at the time of an elevation or lowering of the blood's level of ovarian steroid hormone are almost "trigger mechanisms." One is tempted to consider progesterone a more potent steroid than any of the estrogens as regards endometrial change. Its effect on growth is obvious and more striking than that of physiological amounts of estrogen given in the same period of time. It prevents estrogen-withdrawal regression. Progesterone-withdrawal bleeding is more clear-cut and predictable. But it must be remembered that progesterone is elaborated in quantities measured in terms of milligrams, and estrogens in micrograms. What information we have suggests that the fully mature corpus luteum secretes at least 50 mg. of progesterone daily, and that the recoverable urinary pregnanediol probably represents less than 10 per cent of the progesterone secreted.

Concerning the specific treatment of active uterine bleeding of functional origin, general treatment being taken for granted, estrogenic substance, 6 mg. daily at the very least, will cause endometrial anabolism with resultant cessation of bleeding within a few hours to five days. The anterior hypophysis, already chronically affected by estrogenic hormone, will be further induced to produce whatever luteal stimulation it is able. The ovaries, with their various thecal cysts competing for the available pituitary pabulum, will eventually become atretic. The proliferated, hyperplastic endometrium will become more hyperplastic. With cessation of treatment the status of the patient is not essentially different from what it was before. Hemostasis is all that has been accomplished with these unphysiological amounts of estrogen.

With progesterone, on the other hand, as may be anticipated from the above discussion, not only is hemostasis effected, but the hypophysis and ovaries are put at rest and the endometrium is conditioned to be shed on hormone withdrawal. With the next cycle the hypophysis and ovaries undergo rebound stimulation. It is this rebound stimulation which recommends progesterone as a necessary part of the therapy of any anovulatory situation, whether it be manifest as excessive flowing or amenorrhea. The attainment of this satisfactory state is a matter of dosage and timing of administration. Lack of success

in properly diagnosed cases has been due to underdosage and interrupted instead of consecutive injections. That progesterone-withdrawal bleeding starts 48 to 72 hours after cessation of administration indicates rapid absorption and catabolism.

To mimic the normal cycle ideally, progesterone should be given for 10 days and in doses of at least 10 mg. daily, which are low normal amounts. For rapid hemostasis, 50 mg. at once followed by four doses of 10 to 20 mg. each is a physiological dosage. To satisfy oneself as to what constitute minimal effective doses of progesterone, one may determine how much, injected daily from the 21st to the 26th day of the cycle, will prevent premenstrual staining, or menorrhagia. A total of 50 mg. may be barely adequate, though it will alleviate the accompanying premenstrual tension.

One series of progesterone injections is not likely to remedy the pituitary-ovarian discord manifested in functional bleeding. The number of series, timed so that the last injection is given on the 26th day following the onset of withdrawal flow, must be decided in the individual case. If bleeding starts during injections, one may be certain that a healthy corpus luteum has regressed and that the administered progesterone was not sufficient to prevent menstruation. If bleeding fails to occur within four days of the last injection, a corpus luteum is overriding the given hormone, in which case a period will soon begin or the patient will be found pregnant. If injections happen to be given toward the end of a bout of menorrhagia from a secretory endometrium, a withdrawal flow may fail to ensue.

It is easy to lose sight of the fact that the giving of estrogenic substance, in physiological amounts, involves enhancement of luteal activity. Herein lies the rationale of estrogen therapy for premenstrual tension, *e.g.*, diethylstilbestrol in doses of 0.2 to 0.3 mg. daily from the end of flow until the 26th day. In addition to cyclic progesterone therapy, the same rationale and dosage apply in functional flowing, in which the ovarian production of estradiol probably needs supplementing. Larger doses are interdicted because of the possibility of disturbing the pituitary-ovarian relationship.

The fundamental concept underlying the therapies outlined is comparatively simple, if one keeps in mind the diagram presented above and remembers that diethylstilbestrol has an apparently direct effect upon the pituitary entirely similar to that exerted by the oxidative inactivation products of the naturally occurring estrogens. This concept is based on our laboratory findings. That it is essentially sound is indicated by the improved results in menstrual disorders so treated.

On the basis of the above considerations, it would seem desirable, even urgent, to have available ampules containing 10, 15, 20 and 25 milligrams of progesterone and at a cost that would not cause hesitancy to use them.

OLIVE WATKINS SMITH AND
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Abstracts of
CURRENT ENDOCRINE LITERATURE

Editor: D. A. McGINTY. *Collaborators:* F. A. DE LA BALZE, ISRAEL BRAM, RUCKER CLEVELAND, JOHN W. EVERETT, ANNA FORBES, E. C. HAMBLEN, CHARLES W. HOOKER, R. G. HOSKINS, JANET W. MCARTHUR, THOMAS H. MCGAVACK, J. R. REFORZO-MEMBRIVES, A. E. MEYER, DORIS PHELPS, E. C. REIFENSTEIN, JR., RUTH ST. JOHN, HAROLD WOOSTER, AND J. ZANARTU.

PANCREAS

BOLLMEIER, L. N., AND A. MEYER

The differential diagnosis of glycosuria from diabetes mellitus. *J. Arkansas M. Soc. 41:* 121 (1944).

Review of the literature, both clinical and physiological, reveals evidence that glycosuria may be precipitated by emotional tensions—acutely in otherwise healthy persons, and chronically in patients whose carbohydrate metabolism seems to be disturbed for a decidedly longer period of time and who are considered diabetics. Therefore, it is of great practical importance that reversible emotional glucosuria be differentiated from irreversible diabetes. An early diagnosis will help the physician give the proper treatment, i.e., by psychotherapeutic means with special reference to the environment, diet, and insulin (if required). It is quite possible that the irreversible cases of diabetes are the end results of a functional fluctuating phase, which after a certain duration may lead to irreparable damage of pancreatic function. According to this concept, the course of certain cases of diabetes would follow the same principles which were postulated by Alexander and his co-workers for essential hypertension and peptic ulcers. Emotional glycosuria can be well differentiated from established diabetes by two criteria: (1) fluctuations of the sugar output are influenced more by emotional factors than by carbohydrate intake; (2) emotional glycosuria is a reversible condition. Such findings as Warren's concerning the extreme variability of pathological postmortem findings in diabetes, in the light of this study, find a ready explanation. Emotional glycosuria is a functional condition and consists in the mobilization of stored sugar under influence of emotions. There is a possibility that certain cases of diabetes develop as the advanced phase of a functional disturbance (emotional glycosuria) as a result of an overtaxation of the regulatory mechanisms of sugar metabolism.—*Courtesy Diabetes Abstracts.*

BRUNSCHWIG, A., H. T. RICKETTS AND R. B. BIGELOW. *Surg. Gynec. & Obst. 80:* 252 (1945).

Total pancreatectomy, total gastrectomy, total duodenectomy, splenectomy, left adrenalectomy and omentectomy in a diabetic patient, recovery.

A patient with fairly severe diabetes not due to tumor destruction of the pancreas but of spontaneous origin, was subjected to total pancreatectomy and radical removal of numerous adjacent viscera for carcinoma of the body of the pancreas. The severity of the diabetes was not increased post-operatively. At times the diabetes appeared to be less severe than before total pancreatectomy. When the caloric intake was equivalent to about 2500 (400 grams of carbohydrate), the insulin requirements were 30 units of protamine zinc insulin and 10 regular, daily.—*J.M.*

BRUSH, B. E. AND R. D. MCCLURE

Hyperinsulinism treated by subtotal pancreatectomy. *Ann. Surg. 120:* 750 (1944).

Two additional cases presenting the syndrome of spontaneous hypoglycemia, and in which no islet tumor could be found, are reported. Very satisfactory results followed subtotal resection. Emphasis is again directed to the fact that a large amount of pancreas must be resected in these cases. The study of metabolism of glucose in these patients after they have been on a standard preparatory diet may be of benefit in a diagnostic way and in evaluating the results of treatment. The removal of a

large amount of the pancreas does not seem to cause any subsequent digestive or metabolic disturbance.—*Courtesy Diabetes Abstracts.*

CHAKRABARTY, M. L.

Blood sugar levels in the Bengal famine. *Nature 2:* 519 (1944).

In a communication to *Nature*, the author states that some surprisingly low blood-sugar levels were found; the lowest record was 20 mg. per 100 cc., and this case recovered under treatment. In a normal individual, definite symptoms of hypoglycemia occur when the blood sugar falls below about 70 mg. per 100 cc.; but these starvation cases, although weak and lethargic, with profound depression of all bodily activities, never exhibited any of the typical nervous symptoms of hypoglycemia in spite of their extremely low blood sugars. The absence of hypoglycemia symptoms was probably due to the slow and insidious onset of the hypoglycemia; in such cases of "chronic" hypoglycemia it seems that the nervous system has time to adapt itself to the low sugar level. Biochemical investigation of these cases is proceeding and the main interest so far is the demonstration that life can continue, albeit at a low ebb, with a blood sugar of only 20 mg. per 100 cc.—*Courtesy Diabetes Absiracts.*

DONNELLY, G. L. AND Y. S. PALMER

Functional hypoglycemia. *South Med. & Surg. 106:* 363 (1944).

The clinical entity of hyperinsulinism caused by pancreatic adenomata is rare. A more common condition with the same symptoms and most of the same diagnostic points has been recognized. This condition is a functional disturbance and is not based on any demonstrable changes in the pancreas. Two factors common to most of the patients were: (1) all were working under conditions of increased stress and strain; (2) with one or two exceptions they were nervous, unstable individuals. In this connection it has already been noted that the symptom complex is related to the central nervous system. With laboratory facilities and adequate history, the diagnosis is easy. With reasonable care, the treatment is simple and highly satisfactory.—*Courtesy Diabetes Abstracts.*

JACKSON, ROBERT L. AND C. B. MCINTOSH

Globin insulin with zinc in the treatment of children with diabetes mellitus. *Proc. Cent. Soc. Clin. Res. 17:* 74 (1944).

Six children with diabetes mellitus served as subjects for this study. During the control period, the subjects received a standard diet, designed to meet all nutritional requirements, and given in three equicaloric meals. Amorphous (regular) insulin was administered one-half hour before each meal and once during the night (at 10, 12, or 2 o'clock). The distribution of the day's dosage among the four injections approximated 35, 22, 28, and 15 per cent respectively. In the experimental period, regular insulin was administered one-half hour before the morning and noon meals respectively; the evening and night dosages of the previous regimen (43 per cent of the total) were combined and given as globin insulin with zinc (U-40) one hour before the evening meal. Half the milk was taken from this meal and given three hours later. Otherwise the two regimens were identical. All of the subjects remained under excellent control, and comparative diurnal blood sugars showed no essential change. This study confirms the observation that U-40 globin insulin with zinc has a duration of action of about twelve to fourteen hours.—*Courtesy Diabetes Abstracts.*

JOSLIN, E. P.

Diabetes at the George F. Baker Clinic, New England Deaconess Hospital, Boston, U.S.A. *Caribbean M. J.* 6: 118 (1944).

Various statistics gleaned from diabetic cases treated in a large diabetic clinic are here recorded, and, following their statistical presentation, the methods of treatment employed are given in outline. The average duration of the diabetes already attained in the patients treated during 1941 in the hospital was 7.6 years, or a year and a half more than the total length of life after the onset of the diabetes prior to 1922. Thus, literally, the present group represents a class of diabetics almost nonexistent at the turn of the century and only exceptionally encountered prior to the use of insulin. Diet is the essence of diabetic therapy. Of those diets employed in the clinic, one which contains approximately carbohydrate 150 grams, protein 70 grams, and fat 80 grams is most common. The use of crystalline insulin alone was restricted to 27 cases among 1,640 patients. Protamine zinc insulin alone was given to 732 patients, and, in general, was added in

increasing percentages to all insulins as the age groups advanced.

Ninety per cent of the children and young adults required a combination of both quick-acting and slow-acting insulin; and, conversely, 74 per cent of those reaching 75 years got along comfortably upon protamine zinc insulin alone. The exact dose of protamine zinc insulin is regulated by the number of units necessary to render the urine sugar-free on rising or in a single specimen voided thirty minutes later. Since the introduction of protamine zinc insulin of which the action extends, to some extent, for at least forty-eight hours, the mortality of diabetic coma in this group of patients who die here or anywhere in the world has dropped to 3.3 per cent during 1940–42, as compared with 64 per cent between 1898 and 1914. "We attribute our success in treatment in considerable measure to the cooperation of physicians in this vicinity who recognize cases early and cooperate with us in the administration of insulin before the patient enters the hospital. Without this enlightened support from the medical profession our mortality would not be so low." With insulin, the danger of pregnancy to the mother lessened far more rapidly than the danger to the fetus. Up to January, 1936, the survival rate of the fetus was 55 per cent, but this has increased to 90 per cent among those receiving hormonal treatment.—*Courtesy Diabetes Abstracts.*

LUKENS, F. D. W., AND F. C. DOHAN

Remissions of diabetes mellitus. *Pennsylvania M. J. 48:* 24 (1944).

In recent years the prevention of experimental diabetes and the recovery of early diabetes in animals have been demonstrated. These results have led to a renewed examination of the improvement of diabetes mellitus which occasionally takes place in man. A remission of diabetes means that the diagnosis of diabetes has been made and that, in the absence of complications, the disease has so improved that without insulin treatment the blood sugar stays at a normal level on a diet sufficient to maintain normal weight and strength. Such patients frequently have some abnormality as shown by the glucose-tolerance test, and diabetes will usually manifest itself during an infection. Five hundred seventeen diabetics were surveyed. Of this number, nineteen (3.7 per cent) in whom the diagnosis of diabetes was unquestionable, were on an adequate diet without insulin for at least a short time. In four of them (group I), improvement occurred after dietary treatment alone; in six, it followed active insulin treatment combined with recovery from infection (group II); and in 9 (group III), it resulted from insulin treatment in the absence of complications. These nine remissions in 517 patients give an incidence of 1.7 per cent. From the cases presented and from the literature, it is clear that several factors, often acting simultaneously, may be responsible for the improvement in diabetes mellitus. Such improvement may be due to the restoration of the damaged islands of Langerhans, to a reduction in the requirement for insulin, or to the increased efficiency in the action of insulin. In human diabetes, it is impossible to ascertain the extent to which each or all of these factors are responsible for the improvement in the disease. Certainly there is no direct evidence for restoration of damaged islands, as serial biopsies of the pancreas are not practical in man. However, as this change has been observed in animals, it should be mentioned as an obvious possibility. Of the nineteen patients, seventeen were treated within the first four months of the disease, a finding which stresses the value of early treatment.—*Courtesy Diabetes Abstracts.*

OPPENHEIMER, M. J., AND F. C. MANN

The intralobular pancreatic circulation. *Gastroenterology 3:* 218 (1944).

As a result of study of the intralobular pancreatic circulation after injection of neoprene and corrosion of pancreatic tissue, the following conclusions were drawn: anastomoses exist between intralobular arterioles and also between acinar capillary fields fed by different arterioles within any one lobule; blood may reach acinar capillaries via islets of Langerhans, or directly without passing through insular tissue; the potential intralobular capillary circulation is very large.—*Courtesy Diabetes Abstracts.*

PONZ, J. A., AND D. T. STEVENSON

The effect of Momordica charantia L. ("cundeamor") in diabetes mellitus. *Puerto Rico J. Pub. Health & Trop. Med. 19:* 196 (1943).

In an effort to determine whether the plant possesses blood-sugar-reducing powers, Momordica charantia L., known as "cundeamor" and used in Puerto Rico and other neighboring

countries as a folk remedy in the treatment of diabetes mellitus, was given to seven diabetic subjects and one non-diabetic subject under hospital conditions. It is generally claimed that "cundeamor" controls the symptoms of the disease to the point where dietary restrictions are unnecessary. An alcoholic extract from this plant, claimed to be equally effective, was used in the dosage said to be capable of producing the desired results. A single large dose was also given to two diabetic patients at low glycemic levels and to one non-diabetic subject, after which their blood-sugar levels were determined every half-hour for three and one-half to four hours. The results of this study did not reveal that this particular preparation of the drug possessed any blood-sugar-reducing powers in the dosage used.—*Courtesy Diabetes Abstracts.*

ROOT, H. F.

Thermal burns in diabetes mellitus. *New England J. Med. 232:* 279 (1945).

Two cases of severe thermal burns in diabetics are reported. In both cases there was evidence of an increase in the glucose excretion and the severity of the diabetes. Extensive infection also occurred. When the glucose levels were controlled with insulin the patient improved. The author postulates that the skin may be a source of increased glucose after burns in diabetics.—*L.T.S.*

SEVRINGHAUS, E. L.

High-fat diets in treatment of diabetes. *J. Am. Dietet. A. 20:* 612 (1944).

It would be entirely feasible to standardize the treatment of most diabetics on a diet furnishing about 100 gm. of carbohydrate. This is adequate for all, except the febrile, thyrotoxic, or those with very low thresholds. The caloric intake would then be varied by trial additions of fat until weight control was satisfactory. Protein would be set at, or usually above, 2/3 gm. per kg. of body weight per day. Assuming a 70-gm. protein level, 100 gm. carbohydrate, and 2,800 calories, this would require 235 gm. fat, not at all difficult to eat. To obtain the energy content with as little as 100 gm. fat would require 404 gm. carbohydrate, which would make management of diabetes difficult with or without insulin. If one chose to use 150 gm. fat, the carbohydrate would have to be 291 gm., still a genuinely high carbohydrate diet. The ease of adjusting diet to fit the diabetic seems much greater if the level of carbohydrate is kept below 150 gm. daily. The alleged disadvantages of high-fat allowances have included the increase of ketosis, disproved; the increased risk of arteriosclerosis, which is a bogey; difficulty in digestion, which experience shows is not true, even in cases with chronic cholecystitis. Greater cost of fats, especially in wartime, is offset by the better clinical management and reduced dose of insulin. Finally, the approval of the patients who use the diet shows that high-fat diet can be used for years with comfort, satisfaction, and maintenance of health, so that the pragmatic test for over two decades makes it as defensible as any other.—*Courtesy Diabetes Abstracts.*

SPUEHLER, OTTO

Diabetic glomerulo sclerosis. *Helvet. med. acta. 11:* 27 (1944).

Two hundred eighty-seven cases of diabetes mellitus were observed from 1940 to 1942. Of these, twenty-six were autopsied. Not only clinically but also pathologically, a diagnosis of diabetic glomerulosclerosis could be made. In an additional eight cases a clinical diagnosis of diabetic nephropathy was justified. The pathological-anatomical picture followed three different courses. (1) The glomerulosclerosis of the juvenile diabetic begins as an almost pure lipoid nephrosis with albuminuria, low serum albumin, high blood-cholesterin value, and without increased blood pressure. Only later does the glomerulosclerotic element lead finally to the picture of nephrosclerosis with a high blood pressure, extreme neuroretinitis, and final kidney insufficiency. (2) The process in the middle-aged diabetic is somewhat different, as the vascular sclerotic components are pre-eminent from the beginning and develop simultaneously with the nephrotic syndrome. (3) In diabetes of the aged, nephrosclerosis comes to the foreground, but nephrotic participation does not develop. The cases originally observed by Kimmelstiel and Wilson coincided with the second group of middle-aged diabetics. This diabetic nephrotic disorder is very seldom found in youth, whereas it is more frequent in advanced ages. About a third of all diabetics have glomerulosclerosis. However, the differential diagnosis from genuine arteriosclerotic contraction of the kidneys is becoming more difficult with older people. The blood pressure and the extraordinarily high degree of albuminuria usu-

ally make it possible to diagnose diabetic glomerulosclerosis. The disorder is decidedly progressive, and has in many cases a great similarity to malignant sclerosis. The diabetic lives many years, and develops a gradually increasing benign high pressure with corresponding pathological arteriosclerosis of the kidneys. There can be a sudden change in this phase: the blood pressure rises disproportionately fast, an intense albuminuria appears, the disease takes the form of malignant hypertension. The duration of this development is generally from two to four years. It is this sequence that suggests that an additional process has been superimposed on the gradually developing nephrosclerosis. The fact that it has been impossible up to now to ascertain similar changes outside of diabetes is etiologically of basic significance. A few authors, however, have described cases in which diabetes could not be proved. Such observations should be considered with caution because diabetes was excluded in all cases only on account of lack of glycosuria. On the other hand, with growing contraction of the kidney, the sugar increases. However, not every case of diabetes leads to glomerulosclerosis. From this fact and from the above-mentioned sudden beginning of this disease with the malignant course, it is concluded that diabetes mellitus and its disturbances of metabolism are the basis of the changes of the kidneys; but, besides this, other factors must play a striking part, damages evidently leading to injury of membranes.—*Courtesy Diabetes Abstracts.*

WETZEL, JOHN O.

The eye in diabetes mellitus. *Eye, Ear, Nose and Throat Monthly 23:* 383 (1944).

A review of the literature on the eye complications of diabetes impresses one with the importance of the relation between that disease and the field of the ophthalmologist. Every diabetic should be subjected to a careful ophthalmoscopic examination and his ocular history fully elicited. Such an examination and history-taking is also helpful in detecting arteriosclerosis, which may have been overlooked; and it may be a valuable aid in diagnosing cases permitted to advance to the state of coma.—*Courtesy Diabetes Abstracts.*

PARATHYROID

SPRAGUE, R. G., S. F. HAINES, AND MARSCHELLE H. POWERS

Metabolic effects of parathyroid hormone, dihydrotachysterol, and calciferol in a case of pseudohypoparathyroidism. *J. Lab. and Clin. Med. 30:* 363 (1945).

A patient 33 years old had suffered from pseudohyperparathyroidism (Albright) since the age of 14. Parathyroid hormone had no effect on her condition. Calciferol and dihydrotachysterol both caused a rise in serum calcium and serum phosphorus, a net decrease in fecal calcium, a less marked decrease in fecal phosphorus, and a positive balance for both these substances.—*T.H.McG.*